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The Analysis of Cyanide and its Breakdown Products in Biological Samples

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Cyanide is a toxic chemical that may be introduced into living organisms as a result of natural processes and/or anthropogenic uses (legal or illicit). Exposure to cyanide can be verified by analysis of cyanide or one of its breakdown products from biological samples. This verification may be important for medical, law-enforcement, military, forensic, research, or veterinary purposes. This review will discuss current bioanalytical techniques used for the verification of cyanide exposure, identify common problems associated with the analysis of cyanide and its biological breakdown products, and briefly address the metabolism and toxicokinetics of cyanide and its breakdown products in biological systems.

Keywords cyanide, thiocyanate, 2-amino-2-thiazoline-4-carboxylic acid (ATCA), chemical warfare agent, exposure, toxicology, analytical methods

INTRODUCTION

Cyanide is toxic to humans and animals and exposure can occur in various ways. Many substances are potential sources of cyanide exposure, including edible and non-edible plants (e.g., cassava), industrial operations (e.g., plastics processing), fires, and cigarette smoke. Although the primary natural source of cyanide poisoning is from plants (1–7), other natural sources include volcanoes, bacteria, and fungi (3, 8–12). Anthropogenic sources include malfunctioning catalytic converters, fires involving the burning of plastics, cigarette smoke, and illicit uses of cyanide (e.g., terrorist activities) (13). Additionally, over one million tons of cyanide are manufactured annually worldwide for industrial uses, including chemical syntheses, electroplating, plastics processing, paint manufacturing, gold and silver extraction, tanning, and metallurgy (14). Along with many legal industrial uses of cyanide, multiple illegal uses of cyanide exist, with terrorist acts garnering the most publicity (15). For example, in 1982, cyanide was placed in bottles of Tylenol in the Chicago area, killing seven people (16) and in 1995, cyanide was found in several Tokyo subway restrooms in the weeks following the release of the nerve agent sarin (17). Another illegal

use of cyanide is in the capture of fish for subsequent sale in the live fish trade (18). This practice involves using cyanide at sub-lethal doses to temporarily stun fish, making them easier to catch (19). This practice has been found in a number of countries, and causes irreversible damage to coral reefs (18) by killing the algae that are necessary for coral to survive. It also produces many adverse secondary effects, such as killing smaller fish species (20).

As industrial applications and illicit events involving cyanide increase, the need for rapid, sensitive, and specific analytical methods to assess cyanide exposure will be amplified. The goals for this review are to briefly discuss current bioanalytical techniques used for verification of cyanide exposure and to identify common problems associated with the analysis of cyanide and its metabolites in biological samples. Previous reviews dealing with the analysis of cyanide have also been published and should be consulted to provide a broad view of subject (8, 18, 21–25). This review does not include methods to test for cyanide in environmental or industrial matrices (e.g., surface waters, minerals, process streams).

CYANIDE METABOLISM AND TOXICOKINETICS

Although there are other chemical forms of cyanide (e.g., cyanide ion), it is hydrogen cyanide (HCN) that is the primary toxic agent, regardless of its origin. The toxic effects of cyanide

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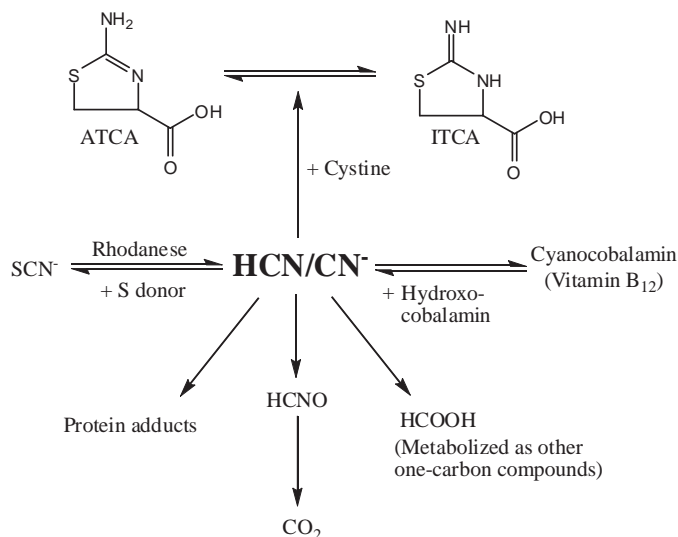


FIG. 1. Human metabolism of cyanide.

can be traced to interference of aerobic metabolism (21, 26, 27). For mammals, fish, and some invertebrates, this occurs when cyanide blocks terminal electron transfer by binding to cytochrome oxidase. For mammals, cyanide ion (CN^-) is acquired through ingestion, while hydrogen cyanide is acquired through inhalation or absorption through the skin. For fish, cyanide is absorbed through the gills or intestine (18), but little else is known about cyanide metabolism in fish. Because the majority of research in this area has been done based on human metabolism, the following text summarizes human metabolism of cyanide.

Once absorbed, cyanide is quickly transferred to the blood and metabolized through a number of processes, as shown in Figure 1. The major pathway for cyanide metabolism is the conversion of cyanide to thiocyanate (SCN^-) in the presence of a sulfur donor (e.g., thiosulfate) (28). This reaction is catalyzed by the enzyme rhodanese (21, 29). About 80% of the initial cyanide dose is converted to thiocyanate (30, 31), which is subsequently excreted in the urine. Other significant metabolic pathways are the conversion of cyanide to 2-amino-2-thiazoline-4-carboxylic acid (ATCA; the tautomeric form of ATCA is 2-iminothiazolidine-4-carboxylic acid – ITCA; Figure 1) by reaction of cyanide with cystine (21, 29, 32, 33), and the reversible reaction of cyanide with hydroxocobalamin to form cyanocobalamin. (Note: Throughout the text, the ATCA/ITCA tautomeric pair will be referred to as ATCA.) It is possible that the production of ATCA over thiocyanate may predominate when sulfur donors become depleted, or where rhodanese is sparse. Other minor pathways for cyanide metabolism include the creation of one-carbon metabolites and protein adducts (i.e., reaction of a chemical species with a protein to form a chemical bond that modifies the parent protein) (21, 34). Each chemical species in Figure 1 could potentially be used as a marker for cyanide exposure.

The toxicokinetics and metabolism of an agent must be considered when an analytical technique is used to determine expo-

TABLE 1
Reported half-lives of cyanide and thiocyanate from acute exposures of cyanide

Cyanide/Metabolite	Species	$t_{1/2}$ (hr)	Reference
Cyanide	Human	0.34–1.00	(46, 252)
	Rat	0.64	(30)
	Pig	0.54	(30)
	Goat	1.28	(30)
Thiocyanate	Human	96–192	(252)
	Rat	5.80	(30)
	Pig	4.95	(30)
	Goat	13.9	(30)

sure to the agent. If the parent toxic agent metabolizes quickly and immediate analysis of the parent agent is not feasible, an alternative metabolic product which is more stable should be chosen as a target for analysis. One consideration for determining which biomarker to target for confirmation of exposure is the half-life of that biomarker. Table 1 lists the half-lives of cyanide and thiocyanate for acute exposures in a number of mammalian species. The half-life of cyanide is short ($t_{1/2} = 0.34\text{--}1.28$ hours), making it difficult to determine exposure to cyanide if significant time has elapsed. Thiocyanate has a longer half-life than cyanide ($t_{1/2} = 4.95\text{--}192$ hours). The half-lives of ATCA and cyanide-protein adducts have not been evaluated, although for cyanide-protein adducts, a half-life similar to that of the parent protein should be expected for a stable adduct (e.g., 20–25 days for human serum albumin) (35, 36). Chronic exposure to cyanide increases the apparent half-life of cyanide and thiocyanate (37–41). The increase in cyanide half-life is likely due to the depletion of sulfur donors (42) because activity of rhodanese over the course of a long-term exposure does not decrease; in fact, it has been shown to increase (43, 44).

As seen by the half-lives of cyanide in a number of mammalian species in Table 1, the toxicokinetics of cyanide is, to some extent, species-dependent. Thiocyanate is also species-dependent, with the estimated half-life of humans being much higher than that of other animals for acute exposures of cyanide (Table 1). Therefore, care must be taken in extrapolating toxicokinetic and metabolic behavior of cyanide between species.

SPECIAL CONSIDERATIONS FOR THE ANALYSIS OF CYANIDE AND ITS METABOLITES

Multiple factors must be considered when choosing which analyte to target for verification of cyanide exposure. Analysis of cyanide or any one of its metabolites has advantages and disadvantages. Table 2 compares cyanide and its metabolites in terms of some factors important for verification of cyanide exposure.

Considering the half-life of cyanide for acute exposure (Table 1), it would be difficult to determine cyanide

TABLE 2
Comparison of cyanide and its metabolites for verification of cyanide exposure

Cyanide/metabolite ^a	Half-lives	Toxicokinetic data	Storage stability	Biological sample ^b	Species ^c
CN	Minutes-hours	Some in a few species	Low	Blood, urine, saliva, tissue, expired air, rumen	Human, fish, cow, mouse, rat, guinea pig, goat, horse
SCN	Hours	Limited	Medium	Blood, urine, saliva, tissue, milk, gastric fluid, cerebrospinal fluid	Human, fish, rat, mouse, pig, goat, horse
ATCA	Unknown	None	High	Blood, urine, feces, saliva, tissue	Human, fish, rat
CN-protein adducts	Presumably days-months	None	Unknown	Blood	Human

^aCN—cyanide, SCN—thiocyanate, ATCA—2-amino-2-thiazoline-4-carboxylic acid.

^bCyanide or the metabolite has been analyzed from this matrix with an analytical method reported in at least one research article or in the laboratories of the authors of this review.

^cCyanide or the metabolite has been analyzed from this species with an analytical method reported in at least one research article or in the laboratories of the authors of this review.

concentrations from biological samples once significant time has elapsed following exposure. Conversely, direct analysis of cyanide may be the only biomarker capable of indicating exposure to cyanide within the initial minutes following exposure (30, 45, 46). The issues that limit analysis of cyanide include the rapid cyanide detoxification processes, the difficulty of establishing steady state cyanide levels with time, and the volatility and nucleophilic properties of cyanide (47, 48). Although traces of cyanide in urine (49–53), saliva (54, 55), and expired air (56–60) have been found, direct analysis of cyanide from these matrices is difficult. Therefore, blood and tissues have been the preferred matrices for cyanide analysis in past studies of cyanide exposure. In tissues, it is difficult to assess rates of cyanide decay in tissues because levels of rhodanese are variable between organs (21), and because free cyanide concentrations are generally low. Although free cyanide may only be available in low concentrations, cyanide may be bound within tissues. Therefore, the possibility of extracting bound cyanide should be considered prior to conducting a specific cyanide analysis technique.

The analysis of the breakdown products of cyanide is a viable alternative to direct analysis. For example, thiocyanate and ATCA have been determined in urine, saliva, tissue, and blood (55, 61–92). Cyanide-protein adducts have been found in human blood proteins (34). The alternative markers of cyanide exposure may be longer-lived (30, 93) and more stable than cyanide (91, 92). Also, correlation of some of these markers to cyanide exposure has been examined (31, 61, 82, 91–96).

The main advantages of analyzing thiocyanate to determine cyanide exposure include the facts that appreciable concentrations of thiocyanate can be found in biological matrices shortly following cyanide exposure and that it has a longer half-life

than cyanide (30, 93). However, thiocyanate is naturally found in biological fluids, and while this is a condition of all cyanide metabolites, thiocyanate levels are relatively high and can be inconsistent (55, 82–88). Large variation in background thiocyanate concentrations makes it difficult to determine low-level cyanide exposure. Also, Ballantyne (97) found that concentrations of thiocyanate in blood varied inconsistently during storage at a number of different temperatures and that analytical recovery of thiocyanate from whole blood was difficult. Large and variable concentrations may indicate that thiocyanate is involved in a number of biological processes in addition to cyanide metabolism (28, 98, 99).

ATCA may be used as an alternative for determination of cyanide exposure. An advantage to using ATCA is that it is stable in biological samples for months at freezing and ambient temperatures (91, 92). Also, ATCA does not metabolize further (21, 31, 100) and therefore, may be a lasting signature of cyanide exposure. However, relatively few studies have been performed to develop analysis techniques for ATCA from biological matrices (61, 89–92) or to evaluate the relationship between ATCA and cyanide exposure (61, 91, 92). It has also been suggested that ATCA is neurotoxic (101, 102) and therefore may contribute to cyanide toxicity. With a greater understanding of its behavior with relation to cyanide exposure, the analysis and characterization of ATCA may prove beneficial.

Recently, cyanide-protein adducts have been discovered (34). If these adducts are stable, they could serve as long-lived markers of cyanide exposure. Currently, this analysis technique requires costly instrumentation and lengthy sample preparation. Even considering these issues, cyanide adducts are extremely promising for providing a long-lived biomarker of cyanide

exposure, especially if a less complex and less costly method of analysis can be developed.

BIOLOGICAL MATRIX CONSIDERATIONS FOR VERIFICATION OF CYANIDE EXPOSURE

Most published methods for determination of cyanide exposure perform the analysis on blood samples. For humans, determination of cyanide exposure has been attempted from blood (34, 37, 45, 49, 51, 55, 59, 82, 95, 103–141), urine (49–53, 64, 142), saliva (54, 55, 64, 70), expired air (56–60), and tissue (post-mortem) (51, 129, 140, 143, 144) samples. Blood may be the most versatile biological sample used to determine exposure to cyanide, because the analyses of cyanide, thiocyanate, ATCA, and cyanide-protein adducts can each be performed on blood samples. Saliva, urine, or tissue may be more appropriate depending on the analytical method and other factors, including ease of obtaining the sample. If determination of cyanide exposure is to be done from non-human species, obviously saliva and urine samples will be difficult to obtain. Therefore, blood and tissues are the most likely samples to be analyzed to determine cyanide exposure. The choice of blood or tissue is determined by a number of factors, including the size of the species. For example, small mice would not have sufficient quantities of blood for some analytical techniques, which makes the analysis of tissue samples indispensable. Another consideration is that rates of cyanide metabolism may be inconsistent between organs because of variable rhodanese and sulfur donor concentrations (21). Therefore, careful selection of tissue, depending on the analyte to be determined, can be extremely important.

THE DETECTION OF CYANIDE AND ITS METABOLITES IN BIOLOGICAL SAMPLES

The determination of cyanide, thiocyanate, ATCA, and cyanide-protein adducts in biological fluids and tissues is useful for forensic, clinical, research, law enforcement, and veterinary purposes. Methods of analysis include spectrophotometry (37, 59, 62, 74, 78, 81, 89, 90, 93, 105, 120, 130, 133, 139, 141, 143, 145–174), fluorescence (49, 51, 89, 115, 121, 132, 142, 175–181), chemiluminescence (77, 109), electrochemistry (18, 56, 63–65, 68, 69, 73, 75, 79, 80, 106, 129, 138, 153, 182–197), gas chromatography (GC) (45, 53, 54, 61, 91, 95, 103–105, 107, 108, 111, 113, 114, 117, 119, 121, 125, 126, 128, 131, 135, 137, 152, 198–212), liquid chromatography (LC) (34, 40, 49, 52, 66, 67, 72, 92, 94, 109, 110, 112, 115, 122, 134, 142, 153, 162, 190–192, 213–228), flow injection analysis (FIA) (64, 109, 191, 193, 215), capillary electrophoresis (CE) (70, 229, 230), and atomic absorption (AA) (231, 232). Choosing from the many available types of analytical methods and biomarkers of cyanide exposure, complicated by numerous discrepancies in the literature between these methods, makes selection of an analytical method daunting. Factors that will influence the initial choice of which biomarker and analytical technique to use are cellular absorption

and detoxification kinetics, sampling and analysis time, sample storage time and conditions, sample matrix, interferences, sensitivity, available instrumentation and equipment, expertise, and cost. In Table 3, some differences between methods listed above are presented.

SAMPLE STORAGE AND PREPARATION

Careful sample preparation and storage of biological samples containing cyanide or its metabolites is a key element to producing accurate results. A significant problem in the analysis of cyanide and thiocyanate is their interconversion, which can occur during sample preparation and storage and leads to inaccurate results (82, 233). The amount of cyanide within the sample can be altered during storage by up to 66% in 14 days, depending on the storage temperature (22, 45, 132, 234–237). There are a number of methods to help prevent artificial formation of cyanide during storage, and if samples containing cyanide are to be stored before analysis, methods to prevent cyanide formation should be considered (see Suggested Procedures for Delayed Analysis of Biological Samples section). For example, Seto et al. (121) demonstrated artificial formation of HCN from thiocyanate in blood and later showed that ascorbic acid prevents artifactual cyanide formation at temperatures below 63°C (234, 238). Also, Sano et al. (122) found that pre-treatment of blood samples with water and methanol was successful in preventing artifactual formation of cyanide from thiocyanate. The artifactual formation or degradation of ATCA does not appear to be a problem as the stability of ATCA in biological samples under a number of storage conditions has been established (91, 92).

Earlier methods of cyanide analysis involved extensive sample preparation in which the sample was acidified (typically with sulfuric or phosphoric acid) and HCN was transferred to alkaline solution by distillation or microdiffusion (37, 49, 82, 95, 110, 132, 139, 147, 168, 173, 179, 239). This served to concentrate the cyanide and to separate it from potential interferences. Buffered hydroxocobalamin (120, 240) or methemoglobin (143) solutions have also been used to capture liberated HCN. This pre-treatment method can be used prior to most analytical techniques for the determination of cyanide and allows the use of analytical techniques that would not normally be useful for biological samples. For example, separation of cyanide by distillation has been used prior to ion-selective electrode (ISE) analysis of cyanide (18). For GC analysis of HCN, the procedure of liberating HCN by acidification (without capture in solution) is extensively used with headspace analysis (45, 103–105, 108, 119, 121, 125, 128, 135, 137, 152, 198, 200, 203, 204, 206, 207, 241). Maseda et al. (95) added a pre-column derivatization step to increase the sensitivity of the GC analysis technique. This technique has also been used prior to headspace solid-phase microextraction (SPME) in which liberated HCN was concentrated on a SPME fiber prior to GC analysis (107, 113). It has also been used in a similar manner for single-drop microextraction

TABLE 3
Analytical methods to determine cyanide and its metabolites in biological fluids^a

Technique ^b	Sub-category	Analyte ^c	Matrices	Sensitivity ^d	Specificity ^d	Sample Size ^d	Capacity ^d	Expertise ^d	Cost ^d	Refs.
UV-Vis	Includes colorimetry and spectrophotometry	CN, SCN, ATCA	Blood, urine, saliva	Low	Low	0.5–1 mL	Medium	Low	Low	(59, 74, 78, 81, 89, 90, 93, 130, 132, 133, 139, 145, 153, 157, 160, 161, 163–166, 169–175, 179)
Luminescence	Fluorescence	CN	Blood	Medium	Medium	0.5–1 mL	Medium	Medium	Low-medium	(51, 132, 152, 176–178, 181)
Electrochemistry	Chemi-luminescence	CN	Blood	Medium-high	Medium	3 μ L	Medium	Medium	Low	(77, 109)
	None	CN, SCN	Blood, saliva, tissue	Medium-high	Medium-high	1–5 mL	Medium-high	Low	Very low-low	(18, 56, 63–65, 68, 69, 73, 75, 79, 80, 106, 129, 138, 153, 182–197)
AA	Indirect AA	SCN	Blood, saliva	Medium-high	Medium	1 mL	Low	Medium	Medium-high	(232)
FIA	None	CN, SCN	Blood, saliva	Medium-high	Medium-high	10–100 μ L	Medium-high	Medium-high	Medium-high	(109, 191, 193, 215)
Biosensor	None	CN, SCN	Blood, urine, saliva	Medium-high	Medium-high	1–5 mL	Medium-high	Low	Very low-low	(146)
LC	RP-HPLC-UV	CN, SCN	Blood, urine	Medium	High	10–100 μ L	High	Medium-high	Medium	(52, 153, 162, 223)
	RP-HPLC-FLD	CN, SCN, ATCA	Blood, urine, saliva	High	High	10–100 μ L	High	High	Medium	(40, 49, 115, 122, 134, 217, 225)
	RP-HPLC-ED	SCN	Urine	Medium-high	High	10–100 μ L	High	Medium-high	Medium	(215, 219, 228)
	RP-HPLC-MS	CN	Blood	Very high	Extremely high	10–100 μ L	High	Very high	High	(110)
	RP-HPLC-MS-MS	CN-protein adduct	Blood	Extremely high	Extremely high	10–100 μ L	Low	Extremely high	Extremely high	(34)
	IC	CN, SCN	Blood, urine, saliva	Medium	High	1–100 μ L	High	High	Medium	(66, 67, 72, 222, 224, 226)
CE	None	SCN	Blood	High	High	1–10 nL	High	High	Medium-high	(70, 218, 226, 229)
GC	GC-NPD	CN, SCN	Blood, urine, saliva	High	Very high	1–10 μ L	Medium	High	High	(45, 53, 113, 114, 121, 126, 131, 135, 137, 152, 198, 202, 207–209)
	GC-ECD	CN, SCN	Blood, urine, saliva	Very high	Very high	1–10 μ L	High	High	High	(95, 103, 119, 125, 128, 200, 205, 211, 212, 241, 246)
	GC-MS	CN, SCN, ATCA	Blood, urine, saliva, tissue	Very high	Extremely high	1–10 μ L	High	High	Very high	(91, 107, 108, 111, 117, 199, 210)

^aThis table is meant to give a general overview of analytical techniques to analyze cyanide and its metabolites along with a general idea about parameters specific to each analysis technique. Parameters of specific methods within a particular analysis technique may be outside of those listed.

^bUV-Vis—ultraviolet visible spectrophotometry, AA—atomic absorption, FIA—flow injection analysis, LC—liquid chromatography, RP—reverse phase, FLD—fluorescence detection, ED—electrochemical detection, MS—mass spectrometry, MS-MS—tandem mass spectrometric detection, IC—ion chromatography, CE—capillary electrophoresis, GC—gas chromatography, NPD—nitrogen phosphorous detector, ECD—electron capture detector.

^cCN—cyanide, SCN—thiocyanate, ATCA—2-amino-2-thiazoline-4-carboxylic acid.

^dThese parameters are related to the general instrumental technique used and not each individual method of analysis.

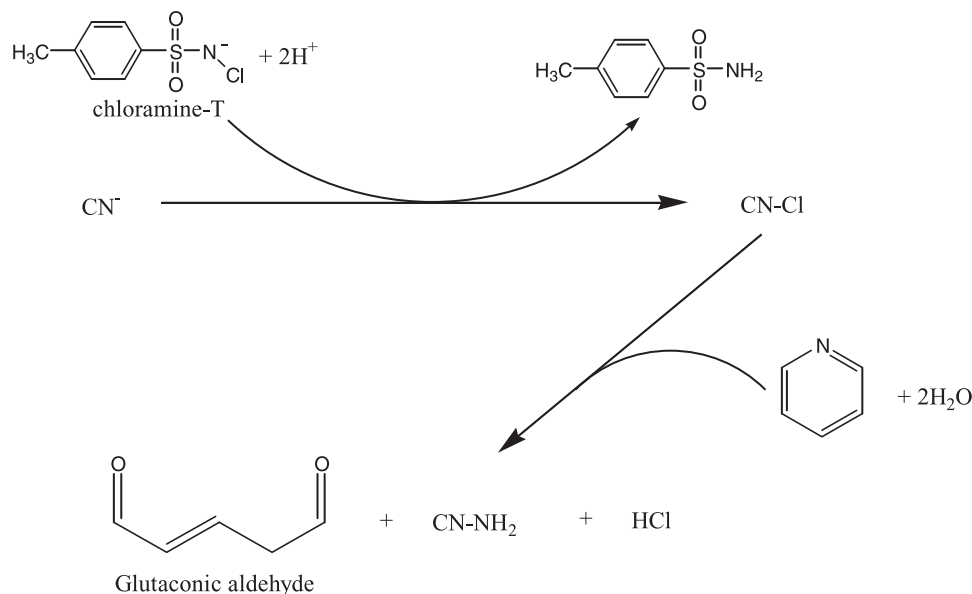


FIG. 2. König reaction for the spectrophotometric analysis of cyanide.

(SDME) with in-drop derivatization and subsequent analysis by CE (229). It should be noted that when using methods that liberate HCN, rubber septa or stoppers can react with gaseous HCN. Therefore, polytetrafluoroethylene septa should be used (48, 95). (Note: It is always necessary to consider the volatility of HCN when working with samples that may have significant concentrations of cyanide and the dangers that it may pose to laboratory personnel.)

Individual pretreatment steps (i.e., derivatizations) are generally necessary for detection of cyanide by spectrophotometry or fluorescence. For example, Lundquist and Sörbo (130) used a modification of the König reaction (Figure 2) for spectrophotometric determination of blood cyanide concentrations by high-performance liquid chromatography (HPLC). Another example is the fluorometric derivatization of cyanide produced from the reaction of cyanide with 2,3-naphthalenedialdehyde (NDA) and taurine (Figure 3). This reaction has been effectively used with HPLC-fluorescence or as a stand-alone fluorescence method to produce highly sensitive methods for the determination of cyanide (49, 50, 110, 112, 115, 122, 134, 142). The derivatization schemes mentioned will be discussed in more depth below along with other derivatization schemes.

Thiocyanate sample preparation normally involves derivatization that is intended to increase a specific detector's response to the ion. While initial sample preparation is not common, ion exchange columns could be used to separate thiocyanate from biological sample components. Thiocyanate is weakly spectrophotometrically active, and therefore is often derivatized with a strong absorber or fluorophore prior to analysis. For example, 3-bromomethyl-7-methoxy-1,4-benzoxazin-2-one has been effectively used (Figure 4) for the fluorometric determination of thiocyanate by HPLC (217). Others have also used variations of the König reaction to produce strong spectrophotometric absorption (157, 162).

Multiple methods have been proposed for the simultaneous analysis of cyanide and thiocyanate. Methods for simultaneous analysis are generally limited to chromatographic methods which involve derivatization. For example, Kage et al. (117) used pentafluorobenzyl bromide (PFBBBr) as the derivatizing agent for simultaneous GC-mass spectrometric (MS) analysis of cyanide and thiocyanate, and Funazo et al. (53) quantitatively methylated cyanide and thiocyanate for GC analysis using a nitrogen-phosphorous detector (NPD). Other authors have used other derivatization techniques for GC analysis of cyanide

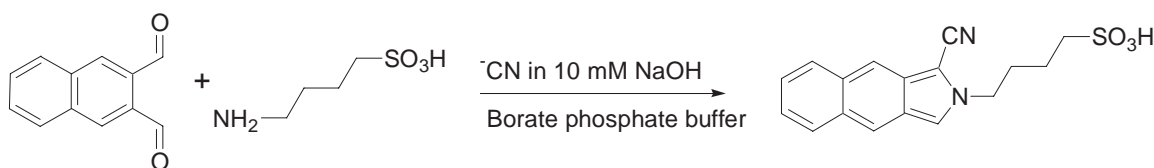


FIG. 3. Derivatization reaction of cyanide with NDA and taurine.

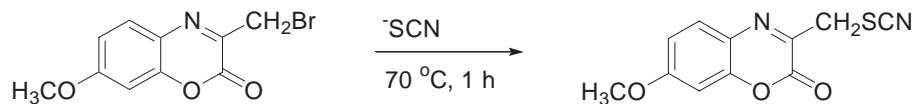


FIG. 4. Derivatization of thiocyanate with 3-bromomethyl-7-methoxy-1,4-benzoxazin-2-one.

or thiocyanate in biological samples (111, 205, 208, 209). LC has also been used for the simultaneous detection of cyanide and thiocyanate. For example, Chinaka et al. (112) used ion chromatography and Toida et al. (134) used HPLC to analyze cyanide and thiocyanate in blood samples.

ATCA has been mainly prepared for analysis using cation exchange solid-phase extraction (SPE) columns and individual pre-treatment steps. Lundquist et al. (92) and Logue et al. (91) both used cation exchange SPE columns to separate ATCA from components of biological samples. Lundquist et al. (92) further purified disulfides from samples by reduction and subsequent separation with another SPE column. Both Bradham et al. (89) and Lundquist et al. (92) heated ATCA in strong basic solution to open the ring structure of ATCA and produce a thiol group. Bradham et al. (89) then used hydroxymercuribenzoate, and subsequently diphenylthiocarbazone, to produce a colored product that was analyzed spectrophotometrically. Alternatively, Lundquist et al. (92) derivatized ATCA (after opening the ring) with a coumarin derivative and then analyzed it by HPLC. After using a cation exchange column (discussed above), Logue et al. (91) prepared ATCA for GC-MS analysis by derivatizing with a silylating agent, thus eliminating the ATCA ring-opening step.

Sample preparation for cyanide-protein adducts involved isolation of the protein of interest with subsequent enzymatic digestion. Fasco et al. (34) used this technique to analyze protein fragments after digestion with trypsin. Although this method was time-consuming and required powerful instrumentation, the authors were able to detect protein-cyanide adducts from the plasma fraction of human blood.

SPECTROPHOTOMETRIC, LUMINESCENCE, AND ATOMIC ABSORPTION METHODS

Early spectrophotometric methods of cyanide analysis from biological fluids were often based on the König synthesis (Figure 2) (60, 168, 169, 171–174, 179, 239). König dye synthesis involves oxidation of cyanide using chloramine-T (139, 147, 157, 160), hypochlorite (130, 133, 176), or bromine water (59, 165, 172, 173) to form a cyanogen halide. The cyanogen halide is then reacted with an aromatic amine (normally pyridine) to produce an aldehyde product that is spectrophotometrically analyzed in the visible region. These methods have adequate sensitivity, but the products are unstable and they lack specificity due to interferences from other chemical species commonly present during the analysis of cyanide, especially thiocyanate and thiosulfate (175). Therefore, these methods often require microdiffusion sample preparation. Modifications have been developed

that yield more stable reagents and increased precision for this type of reaction (59, 93, 133, 147, 163–166).

Spectrophotometric analysis of thiocyanate is often a variation of the König reaction described above. Hypochlorite and thiocyanate react to form the cyanogen chloride, then either pyridine-malononitrile (161) or barbituric acid-pyridine reagent (130, 157, 159, 162) can be used as coupling agents. Cyanogen chloride can also be combined with isonicotinic acid to produce a glutamic aldehyde. Condensation of this aldehyde with two molecules of 1,3-dimethylbarbituric acid produces a dye which can be analyzed spectrophotometrically (153). Other early methods of thiocyanate analysis (170) involved oxidation of the thiocyanate to hydrogen cyanide, with aeration into alkaline solution, permitting the determination of cyanide as described above for the König reaction. The modified König reaction was also applied to the simultaneous analysis of cyanide and thiocyanate (52, 134, 172, 173). Nagashima (160) used the differences in the rates of the reaction of cyanide and thiocyanate with chloramine-T and variations in pH dependence for the simultaneous spectrophotometric determination of cyanide and thiocyanate.

ATCA has also been analyzed spectrophotometrically. Bradham et al. (89) analyzed ATCA from urine as described previously. Limitations for this method include interference from a number of species (including cyanide ions) and lengthy sample preparation.

Fluorescence (49, 51, 115, 132, 175–178, 181) methods have been applied to the determination of cyanide in biological fluids. As with spectrophotometric methods, fluorescence methods normally require extraction techniques to isolate cyanide and eliminate interferences. A number of sensitive fluorometric assays to determine cyanide, free of thiosulfate interference, have been developed with greater sensitivity than spectrophotometric methods (51, 176, 177). One specific fluorometric method for the analysis of cyanide is the reaction of cyanide with NDA and taurine to create a highly fluorescent isoindole (Figure 3) (49, 115). A chemiluminescence method has also been developed for the analysis of cyanide from whole blood (109). For this method, acidification and distillation was used to separate cyanide from interfering whole blood components and a microchip-based reactor was used to mix sample preparation reagents and produce chemiluminescence.

Although non-chromatographic fluorescence methods for the determination of thiocyanate, ATCA, or cyanide-protein adducts have not been published, the possibility exists to use such methods for ATCA and thiocyanate. For instance, 3-bromomethyl-7-methoxy-1,4-benzoxazin-2-one (Figure 4) and coumarin dyes

have been used effectively for the HPLC-fluorometric determination of thiocyanate (217) and ATCA (92), respectively.

AA methods are indirect methods of cyanide or thiocyanate analysis. A metal complex is formed with the thiocyanate or cyanide which subsequently precipitates or is extracted into an organic solvent. This metal complex is then analyzed by AA spectrometry. For example, cyanide has been analyzed indirectly by complexing with an iron(II)-phenanthroline, extracting in chloroform, analyzing iron by AA, and directly relating the iron concentration to the cyanide concentration (242). Chattaraj and Das (232) used AA spectrometry for the analysis of thiocyanate in biological fluids by forming a complex with thiocyanate and copper and determining the copper signal. This technique has not been used to determine ATCA or cyanide-protein adducts.

ELECTROCHEMICAL, ION-SELECTIVE ELECTRODE, AND BIOSENSOR METHODS

Many electrochemical methods for the detection of cyanide and thiocyanate exist, but relatively few of these methods have been applied to their analysis in biological samples (18, 56, 63–65, 68, 69, 73, 75, 79, 80, 106, 129, 138, 153, 182–197, 199, 243). The benefits of using electrochemical methods are high sensitivity and quick analysis time. However, they can be subject to multiple interferences from many organic and inorganic ions, including S^{2-} , ClO_4^- , NO_2^- , N_3^- , and I^- (129, 188). Electrochemical methods can also be hampered by narrow working concentration ranges and may require large sample sizes (185, 187). Westley and Westley (129) used a silver rotating disk electrode and a dropping mercury electrode for the voltammetric determination of cyanide and thiocyanate in biological samples, including plasma, tissue, and whole blood. Electrochemical detection can also be used with ion chromatography for the analysis of cyanide and thiocyanate (190).

Polymeric membrane-based ISEs have been developed to address some of the issues limiting electrochemical analysis of cyanide and thiocyanate (23, 64, 65, 69, 73, 75, 79, 80, 106, 182–184, 186–189, 194, 243–245). ISEs can exhibit rapid response, high sensitivity, wide linear range, low cost, and they are usually simple to operate. The polymeric membrane in ISEs contains an ion carrier that interacts selectively with the analyte. This interaction is often enhanced by the use of a metal in the ion carrier that strongly interacts with the analyte ion. Although many ISEs exist, they are somewhat limited for anions compared with cations (188). ISEs have been developed for cyanide analysis, but few have been used for analysis of cyanide from biological samples. This is due to the interaction of multiple ions and biological materials present in biological samples. If these interferences can be removed, then ISE methods could be used for the determination of cyanide from biological samples. In fact, the standard method for the analysis of cyanide from fish tissues is based on an ISE (245). For this method, tissue samples are prepared by homogenizing, acidifying, and distilling internal organs of fish species. Cyanide present in the homogenized tissue is converted to HCN and captured in an alkaline solution

following distillation. A cyanide ISE is then used to analyze for cyanide based on its interaction with silver. Another ISE test using gold disc electrodes coated with a sulfonated tetrafluoroethylene copolymer was recently developed by Lindsay and O'Hare for the analysis of cyanide in blood without sample pre-treatment (106).

Thiocyanate, due to its lipophilicity, is well-suited for ISE analysis and has been successfully analyzed by ISEs (23). ISEs have been developed that exhibit good agreement with ion chromatography (194) and spectrophotometry (186–189), and have been used to analyze thiocyanate from plasma (194), urine (64, 65, 68, 69, 73, 79, 80, 184, 186–189, 243), and saliva (64, 66, 68, 69, 73, 79, 80, 182, 183, 186–188). For example, ISEs based on crystal violet or methylene blue and a selective polymeric membrane containing a nickel(II)-azamacrocyclic complex showed excellent selectivity and sensitivity and were successfully used for the analysis of thiocyanate in urine and saliva (187, 194). Other electrodes which have shown good selectivity for thiocyanate in biological samples include a polyvinyl chloride (PVC) membrane electrode based on a nickel-hexaazacyclotetradecane derivative (186), a PVC membrane electrode with an unsymmetrical nickel(II) macrocyclic complex as an ion carrier (188), and a graphite electrode based on iron phthalocyanine membranes (189).

Many biosensors (small detection devices normally based on biological activity toward an analyte) exist for the determination of cyanide, including microbial cyanide sensors, sensors based on the enzyme inhibition of cyanide, and sensors based on cyanide degrading enzymes. Most biosensors have the advantage of being portable, inexpensive, easy to use, and can have high selectivity. Limitations of biosensors include degradation of biological components that make up these sensors, inconsistent electrochemical signals, and difficulty producing sufficient quantities and activities of enzymes or microorganisms on which these sensors depend. Most of the biosensors developed for cyanide analysis have not been applied to the analysis of biological samples, although a biosensor system for the determination of cyanide in fish was recently described (146). For this method, cyanide hydrolase was used to convert cyanide into formate and ammonia. The formate was detected and converted to CO_2 with a formate biosensor, which produced NADH from NAD^+ . A Clark electrode was used to monitor the consumption of oxygen from the oxidation of NADH back to NAD^+ (18).

Currently, biosensors have not been applied to thiocyanate detection from biological samples. Also, electrochemical and biosensor methods have not been used to analyze for ATCA and cyanide-protein adducts from biological samples.

LIQUID CHROMATOGRAPHY, CAPILLARY ELECTROPHORESIS, AND FLOW INJECTION ANALYSIS

The complex nature of biological matrices, the small concentrations of cyanide and its metabolites, and the high number of species that interfere with spectrophotometric, luminescent, and electrochemical methods have often necessitated analysis

of cyanide and its metabolites by more powerful methods. LC techniques can determine trace amounts of an analyte and can efficiently separate analytes from interfering components in the matrix. Chromatographic techniques, both liquid and gas, also have the ability to simultaneously analyze for cyanide and thiocyanate. For these reasons, LC methods have gained popularity in analysis of cyanide and its markers in biological samples.

Two types of LC have been used to analyze cyanide: reverse-phase high-performance liquid chromatography (RP-HPLC) (34, 40, 50, 52, 92, 94, 110, 115, 122, 134, 142, 217, 221, 225, 228) and ion chromatography (IC) (112, 162, 190–192, 213, 216, 218–220, 222–224, 226). RP-HPLC methods are common, but generally require pre-treatment steps for each anion or multiple post-column reagents. IC methods are common for thiocyanate analysis but cyanide is not normally analyzed with this technique. Some common detectors used in the LC analysis of cyanide or its metabolites include spectrophotometric (52, 72, 94, 153, 162, 223), fluorescence (40, 49, 115, 122, 134, 142, 217, 225), electrochemical (193, 215, 219, 228), and MS (34, 110) detection. CE (230) is a related method of analysis that involves initial separation of a sample and subsequent analysis.

Several groups have adapted the spectrophotometric detection of cyanide and thiocyanate in blood and urine based on the König reaction (Figure 2) to RP-HPLC (153, 162, 223, 227). This reaction has also been used for HPLC with fluorometric detection. Toida et al. (134) analyzed cyanide in blood at picomole levels with HPLC and fluorometric detection by using a variant of the König reaction, replacing pyridine with pyridine-barbituric acid. Fluorescence detection was also used for the determination of cyanide in human erythrocytes, whole blood, and urine using RP-HPLC and pre-column derivatization with NDA and taurine (Figure 3) (50, 122). A number of other fluorometric HPLC methods have been developed for the analysis of cyanide and its metabolites from biological fluids (112, 115, 217, 225).

Detection of thiocyanate and simultaneous detection of cyanide and thiocyanate have been accomplished using RP-HPLC with fluorometric detection. Tanabe et al. (225) used HPLC with fluorometric detection to determine thiocyanate in saliva and serum based on the reduction of cerium (IV) to fluorescent cerium (III). Using pentafluorobenzylbromide as derivatizing agent, Liu and Yun (94) simultaneously determined cyanide and thiocyanate in blood and milk. Tracqui et al. (110) used microdiffusion sample preparation followed by derivatization with NDA and taurine for HPLC-MS analysis of cyanide in blood.

ATCA and cyanide-protein adducts have also been analyzed with RP-HPLC (34, 92). Lundquist et al. (92) used HPLC with fluorometric detection for the determination of ATCA in urine. Although this method was time consuming, ATCA was detected in the urine of smokers. Fasco et al. (34) used RP-HPLC with tandem MS detection to analyze cyanide-adducted proteins from human plasma. The method involved isolation and enzymatic digestion of cyanide-adducted human serum albumin.

A limited number of IC methods have been developed for analysis of cyanide (112). Conversely, multiple IC methods have been used to analyze biological fluids for thiocyanate (72, 112, 162, 190–192, 213, 216, 218–220, 222–224, 226). Chinaka et al. (112) used NDA derivatization of cyanide with an ion exchange column for the simultaneous determination of cyanide and thiocyanate. Lundquist et al. (162) used an ion exchange column with visible spectrophotometric detection for the determination of thiocyanate in serum and urine. Connolly et al. (213) used ion interaction LC with UV detection to analyze thiocyanate in urine. ISEs have also been used to detect thiocyanate in urine following IC (190).

Another type of LC that involves both RP and ionic interactions is called ion-pairing chromatography. With ion-pairing chromatography a column modifier is added to the mobile phase to create an ionic stationary phase. Authors using this type of chromatography have coated RP-HPLC columns with ion-pairing agents to mainly analyze thiocyanate. Examples of this type of analysis include RP-HPLC columns coated with cetyldimethylamine (222, 224), a zwitterionic micelle (192), and bovine serum albumin (216). Brown et al. (40) used a cetylpyridinium coated RP column to create an ion-exchange column for the analysis of thiocyanate from rainbow trout plasma in a pharmacokinetic study of thiocyanate exposure.

CE and FIA are not chromatographic methods because they do not utilize a stationary phase but they are similar in that small precise volumes of sample are added to a continuously flowing carrier stream. Moreover, CE is also used to separate the components of a sample prior to detection and both CE and FIA normally use LC detectors. CE has been used successfully for the analysis of cyanide (229) and thiocyanate (70, 230) in biological fluids. Glatz et al. (230) analyzed blood, urine, and saliva samples for thiocyanate with CE and spectrophotometric detection, with no sample preparation aside from dilution. Jermak et al. (229) used CE for determination of cyanide after headspace SDME with in-drop derivatization. CE methods for cyanide, ATCA, and cyanide-protein adducts have not been suggested. A number of FIA methods have been proposed to analyze for cyanide biomarkers in biological samples (64, 109, 191, 193, 215). For example, Cookeas and Efstathiou (193) successfully determined thiocyanate in saliva with FIA and ISE detection using a cobalt-phthalocyanine modified carbon paste electrode.

GAS CHROMATOGRAPHY

One of the most common methods for analysis of cyanide, thiocyanate, and more recently ATCA is gas chromatography. Common detectors used for analysis of cyanide or its metabolites are the electron capture detector (ECD) (95, 103, 119, 125, 128, 200, 205, 211, 212, 241, 246), nitrogen-phosphorus detector (NPD) (45, 53, 65, 105, 113, 114, 117, 121, 126, 131, 135, 137, 152, 202, 207–209), and MS detector (61, 91, 107, 108, 111, 117, 199, 210). Although most of these methods are used for detection of cyanide in blood, some groups have applied GC

techniques for other biological matrices and for the detection of thiocyanate and ATCA.

For the detection of cyanide from biological matrices, no specific derivatization is necessary as HCN is volatile. Therefore, the most common pre-analysis step in GC analysis of cyanide is sampling of cyanide from the sampling of the headspace following acidification of the sample. Either equilibrium or dynamic headspace methods can be used to prepare a sample for GC analysis (45, 103–105, 108, 113, 119, 121, 125, 128, 135, 137, 152, 199, 203, 204, 206, 207, 241). Another pre-analysis step for cyanide analysis is cryogenic oven trapping, which has been used to trap liberated HCN and produce high resolution and sensitivity (114, 202). Because thiocyanate and ATCA are non-volatile, they require chemical modification (i.e., derivatization) to allow analysis by GC. Also, chemical modification is necessary for analysis of cyanide by ECD (because of its poor response to underivatized cyanide) or for simultaneous analysis of cyanide and thiocyanate. Therefore, a number of pre-treatment steps have been developed to facilitate the analysis of cyanide, thiocyanate, and ATCA by GC.

In GC analysis, the NPD permits the sensitive and specific detection of nitrogen- or phosphorous-containing compounds, and has been used for the detection of cyanide and thiocyanate in blood, plasma, urine, and saliva (45, 53, 105, 113, 114, 117, 121, 126, 131, 135, 137, 152, 198, 202, 207–209). However, this detector may be unstable at times, and is less sensitive than some other types of GC detectors. This has led to the creation of analytical methods that take advantage of more stable and sensitive detectors such as the ECD and MS. PFBBR is most commonly used for the derivatization of cyanide, especially for ECD detection (117, 118, 247, 248). The conversion of HCN into cyanogen chloride by choramine-T oxidation (Figure 2) has also been used for GC-ECD analysis (95, 103, 119, 212).

One of the most sensitive methods for analysis of cyanide, thiocyanate, and ATCA is GC-MS (54, 61, 91, 107, 108, 111, 199, 201, 203, 210). With MS detection, stable isotope internal standards (e.g., ^{13}CN , C^{15}N , or $^{13}\text{C}^{15}\text{N}$) can be used to correct for matrix effects common to cyanide and cyanide metabolites. This can eliminate the need for standard addition techniques and matrix matching. Murphy et al. (199) and Dumas et al. (108) used a stable isotope internal standard with GC-MS and head space analysis to analyze cyanide concentrations from blood. GC-MS has also been used to analyze cyanide and thiocyanate simultaneously (54, 117). Logue et al. (61, 91) analyzed ATCA by GC-MS in plasma and urine by first converting the non-volatile metabolite into a volatile form using trimethylsilyl-trifluoroacetamide. Cyanide-protein adducts have not been analyzed by GC-MS.

SUGGESTED PROCEDURES FOR DELAYED ANALYSIS OF BIOLOGICAL SAMPLES

As discussed earlier, because of the rapid detoxification of cyanide from blood samples, a sample for cyanide analysis should be collected quickly after exposure and analysis

should be performed as soon as possible. However, if analysis of cyanide cannot be performed quickly and storage of biological samples is necessary, the following should be considered:

1. *Volatility and nucleophilicity of cyanide.* HCN is volatile and the cyanide ion is nucleophilic. Tightly sealed vials, low temperatures, high pH (i.e., $\text{pH} > 10.5$), and the addition of preserving agents are common procedures that have been used to prevent loss of cyanide. Storing samples at low temperatures is extremely important to reduce evaporative loss and slow biochemical reactions. However, there are many discrepancies in the literature when evaluating the stability of cyanide in biological fluids under various conditions (82, 133, 175, 237, 249). Generally, nucleophilic losses are reduced by adding sequestering agents (e.g., hydroxocobalamin) or chemicals that produce sequestering agents (e.g. sodium nitrite to produce methemoglobin) (130, 133, 250). One method found to improve cyanide stability is the addition of silver ions to biological samples (133).
2. *Cyanide concentration varies in biological components.* Cyanide in blood primarily resides in erythrocytes (red blood cells) (48, 133, 139, 236, 251) by binding to methemoglobin, forming cyanomethemoglobin. Cyanide may also be present in plasma, especially if cyanide concentrations exceed hemoglobin concentrations (133, 139). To ensure accurate cyanide concentrations when analyzing blood, collection containers that contain anti-coagulants (e.g., heparin) should be used to prevent clotting, and analysis of whole blood or both plasma and red blood cells should be performed. Analysis of cyanide from tissues requires knowledge of the behavior of cyanide in specific organs since the enzyme that catalyzes conversion of cyanide to thiocyanate has highly variable concentrations depending on the organ. Therefore, in specific organs, there will be little to no cyanide because of extremely fast metabolism to thiocyanate.
3. *Potential for cyanide formation during storage.* Artfactual formation of cyanide may also occur in biological samples depending on storage conditions (132, 235–237). It has been suggested that oxyhemoglobin (234), thiocyanate oxidase (235, 236), and white blood cells (132) may oxidize thiocyanate to cyanide and these reactions are dependent on the temperature and pH of the sample. Microorganisms may also be responsible for cyanide production and low temperature storage will help to eliminate their growth (235).

These considerations are common to all the analytical methods for analysis of cyanide from biological samples and certainly contribute to discrepancies in similar studies in the literature. Additionally, production and transformation of cyanide must be considered when interpreting results for post-mortem cyanide analysis (140, 252–255).

Some of the same issues must also be considered for thiocyanate, as a number of problems with storage of thiocyanate samples have been found (97). This may be due to the interconversion of thiocyanate and cyanide over time and the removal and production of thiocyanate by biological processes other than

TABLE 4
Concentrations of cyanide found for smokers vs. nonsmokers

Biological matrix	Non-smoker (μM)	Smoker (μM)	Analytical method ^a	Reference ^b	Notes ^c
Blood	0.518 ± 0.037 (7)	0.597 ± 0.062 (6)	IC-FLD	(112)	Whole blood
	2.1 (6)	6.6 (11)	GC-ECD	(136)	Whole blood
	0.13 ± 0.08 (10)	0.33 ± 0.12 (5)	UV-Vis	(133)	Whole blood
	0.24 ± 0.22 (10)	0.68 ± 0.20 (5)	UV-Vis	(133)	Red blood cells
	0.02 ± 0.02 (10)	0.03 ± 0.02 (5)	UV-Vis	(133)	Plasma
	1.2 (10)	1.8 (10)	UV-Vis	(259)	Whole blood
	0.098 ± 0.036 (8)	0.125 ± 0.035 (5)	Fluorescence	(132)	Whole blood; study separated by time post-smoking; concentration reported here is referred to as "basal"
	0.14 ± 0.01 (3)	0.35 ± 0.09 (4)	RP-HPLC-FLD	(134)	Red blood cells
	0.466 ± 0.072 (10)	0.705 ± 0.112 (10)	RP-HPLC-FLD	(122)	Red blood cells
	Not detected (5)	0.058 ± 0.030 (5)	Fluorescence	(49)	Whole blood
	0.02 (1)	0.06 ± 0.03 (5)	IC-ED	(49)	Whole blood; only one value reported above detection limit for non-smokers
	0.59 ± 0.23 (10)	1.5 ± 0.6 (14)	UV-Vis	(237)	Whole blood; standard deviations reported here are calculated from standard errors
	2.9 ± 2.4 (29)	6.8 ± 4.2 (27)	GC-ECD and UV-Vis	(260)	Whole blood; the cyanide concentrations reported were not separated by analytical method
	0.17 ± 0.04 (20)	0.27 ± 0.07 (20)	GC-NPD	(55)	Whole blood
	0.335 ± 0.008 (31)	0.548 ± 0.123 (15)	RP-HPLC-FLD	(88)	Whole blood; measured as mol/g of hemoglobin and estimated whole blood concentration
Urine	0.084 ± 0.032 (8)	0.215 ± 0.084 (8)	RP-HPLC-FLD	(50)	
	0.80 (10)	1.2 (10)	UV-Vis	(259)	Average concentration over a twenty-four hour period
	0.050 ± 0.022 (4)	0.28 ± 0.02 (5)	Fluorescence	(49)	Non-smoker value reported here is for the four cyanide concentrations reported
Saliva	0.054 ± 0.023 (5)	0.30 ± 0.02 (5)	IC-ED	(49)	
	11.8 ± 7.2 (10)	No Data	GC-MS	(54)	
	0.38 ± 0.26 (20)	0.66 ± 0.52 (20)	GC-NPD	(55)	

^aSee method notes in Table 3.

^bExcluded Pettigrew and Fell (82) because concentrations of cyanide in smoker blood were below non-smoker blood and relative errors were very large.

^cThe term "plasma" refers to serum or plasma.

cyanide detoxification (28, 99). It has been suggested that ATCA is not involved in other biological processes and has been found to be stable during storage (21, 31, 91, 92, 100). While ATCA may not have the storage issues of cyanide and thiocyanate, there is little known about its toxicokinetics, which currently limits

its use as a biomarker for cyanide exposure. Cyanide-protein adducts have only recently been discovered. Therefore, little is known about the toxicokinetics and stability of these adducts, and only one analytical technique has been developed to analyze these adducts (34).

TABLE 5
Concentrations of thiocyanate found for smokers vs. nonsmokers

Biological matrix	Non-smoker (μM)	Smoker (μM)	Analytical method ^a	Reference	Notes ^b
Blood	7.5 \pm 3.3 (13)	26 \pm 9 (18)	UV-Vis	(97)	Whole blood
	33 \pm 13 (13)	116 \pm 39 (18)	UV-Vis	(97)	Plasma
	25.2 \pm 10.8 (7)	No Data	UV-Vis	(170)	Plasma
	6.9 (10)	17.2 (10)	UV-Vis	(259)	Whole blood; no standard deviations were reported
	33.8 (6)	122 (8)	UV-Vis	(86)	Plasma; no standard deviations were reported
	55.3 \pm 12.0 (20)	94.7 \pm 36.2 (24)	UV-Vis	(261)	Plasma; The averages and standard deviations were combined for light and heavy smokers
	21.6 \pm 6.2 (107)	145.5 \pm 50.1 (108)	UV-Vis	(87)	Plasma; values are for non-smoker and an average of the two "inhaling smoker" groups reported
	50.7 (100)	122.9 (94)	UV-Vis	(85)	Plasma; averaged a number of passive smoke exposure groups for non-smoker value
	42.5 \pm 17.1 (20)	No Data	UV-Vis	(162)	Plasma
	36.2 \pm 7.4 (100)	62.6 \pm 19.3 (86)	UV-Vis	(84)	Plasma
	33.5 \pm 25.4 (20)	111.2 \pm 92.1 (20)	UV-Vis	(55)	Plasma
	33 \pm 26 (101)	158 \pm 51 (92)	GC-NPD	(208)	Plasma
	87.5 \pm 33.2 (3)	196.4 \pm 44.9 (3)	CE	(230)	Plasma
	4.83 \pm 0.47 (31)	8.94 \pm 1.04 (15)	RP-HPLC-UV	(88)	Plasma
	33 \pm 15 (181)	109 \pm 47 (187)	IC-UV	(262)	Plasma
	62 \pm 19 (24)	161 \pm 43 (26)	FIA	(263)	Plasma
	45.8 \pm 17.1 (9)	86.4 \pm 32.5 (11)	UV-Vis	(82)	Plasma
	36 \pm 28 (10)	65 \pm 74 (20)	UV-Vis	(93)	Plasma; standard deviation reported here is calculated from reported standard error of the mean
	10.6 \pm 5.0 (7)	55.8 \pm 22.3 (6)	IC-UV	(112)	Whole blood
	11.7 \pm 5.5 (40)	83.1 \pm 51.7 (13)	IC-UV	(224)	Plasma; value reported for non-smokers is average of individual data reported
	14.1 \pm 4.6 (3)	52.1 \pm 37.1 (4)	RP-HPLC-FLD	(134)	Red blood cells
	19.8 \pm 7.9 (3)	57.3 \pm 36.2 (4)	RP-HPLC-FLD	(134)	Plasma
	8.1 \pm 3.3 (10)	27 \pm 9 (14)	UV-Vis	(237)	Whole blood; standard deviations reported here are calculated from standard errors
Urine	24.1 (6)	141 (8)	UV-Vis	(86)	No standard deviations were reported
	28.7 \pm 14.7 (20)	No data (20)	UV-Vis	(162)	Reported in mg/24 hr; Value reported here calculated from normal human urine output (ca. 1.5 L/24 hr)
	262 \pm 105 (3)	No data	UV-Vis	(170)	Averaged a number of passive smoke exposure groups for non-smoker value
	75.1 (100)	154.9 (94)	UV-Vis	(85)	

(Continued on next page)

TABLE 5
Concentrations of thiocyanate found for smokers vs. nonsmokers (*Continued*)

Biological matrix	Non-smoker (μM)	Smoker (μM)	Analytical method ^a	Reference	Notes ^b
	240 \pm 35 (4)	660 \pm 162 (4)	UV-Vis	(243)	
	233 \pm 36 (4)	650 \pm 157 (4)	ISE	(243)	
	250 \pm 50 (1)	760 \pm 80 (1)	UV-Vis	(186)	Averaged analysis of 5 urine samples from one smoker and one non-smoker
	230 \pm 40 (1)	770 \pm 50 (1)	ISE	(186)	Averaged analysis of 5 urine samples from one smoker and one non-smoker
	41–48	170–4,500	ISE	(264)	1 non-smoker group and 4 smoker groups; no data given on number of participants in each group; range and not average reported
	14 (211)	16 (305)	UV-Vis	(84)	Numerical data for urine not reported; estimated concentration from Figure 5
	14 (10)	71 (10)	UV-Vis	(259)	Average concentration over a 24-hr period
	24 \pm 25 (29)	65.3 \pm 40 (15)	GC-MS	(201)	Only reported concentration ranges in paper; Estimated median values and standard deviation from Figure 3 and ranges reported for value reported here
	No data	430 (1)	IC-ED	(190)	Only 1 smoker sample used for analysis
	260 \pm 60 (1)	730 \pm 60 (1)	ISE	(187)	The standard deviation reported is for replicate measurements on the same sample
	84.0 \pm 39.9 (3)	216.5 \pm 49.2 (3)	CE	(230)	
	32 (1)	240 \pm 71 (2)	UV-Vis	(188)	Only 1 non-smoker tested; the smoker value reported here excludes a diabetic smoker
	30 (1)	240 \pm 56 (2)	ISE	(188)	Only 1 non-smoker tested; the smoker value reported here excludes a diabetic smoker
	60.4 \pm 17.4 (6)	171.0 \pm 42.1 (6)	HPLC-UV	(52)	
	112 (1)	338 \pm 165 (3)	IC-UV	(213)	Average of reported “medium” and “heavy” smokers; only 1 non-smoker tested
	360 \pm 30 (1)	770 \pm 30 (1)	ISE	(75)	Appears to be 1 smoker and 1 non-smoker with 5 replicates each.
	340 \pm 30 (1)	730 \pm 40 (1)	UV-Vis	(75)	Appears to be 1 smoker and 1 non-smoker with 5 replicates each.
	125 \pm 100 (10)	1433 \pm 300 (30)	UV-Vis	(64)	Only concentration ranges given; combined 3 test groups for the smokers and averaged

(Continued on next page)

TABLE 5
Concentrations of thiocyanate found for smokers vs. nonsmokers (*Continued*)

Biological matrix	Non-smoker (μM)	Smoker (μM)	Analytical method ^a	Reference	Notes ^b
Saliva	138 \pm 200 (10)	1473 \pm 283 (60)	ISE	(64)	Only concentration ranges given; combined 6 test groups (both batch and FIA) for the smokers and averaged
	42.9 \pm 42 (7)	219 \pm 172 (9)	IC-UV	(222)	Values reported here are for male and female non-smokers combined; standard deviations estimated from relative deviations in the reference
	350 (6)	1304 (8)	UV-Vis	(86)	No standard deviations were reported
	15.5 \pm 6.9 (211)	17.2 \pm 5.2 (305)	UV-Vis	(84)	
	1550 (135)	2550 (12)	UV-Vis	(149)	Reported data in terms of concentration ranges for smokers and non-smokers; estimated median concentration reported here
	560 \pm 50 (1)	1710 \pm 70 (1)	UV-Vis	(186)	Averaged analysis of 5 saliva samples from 1 smoker and 1 non-smoker
	580 \pm 40 (1)	1690 \pm 50 (1)	ISE	(186)	Averaged analysis of 5 saliva samples from 1 smoker and 1 non-smoker
	620 \pm 50 (1)	1820 \pm 80 (1)	ISE	(187)	The standard deviation reported is for replicate measurements on the same sample
	1,330 (100)	2,450 (94)	UV-Vis	(85)	Averaged a number of passive smoke exposure groups for non-smoker value
	643 \pm 213 (10)	No Data	GC-MS	(54)	
	542 \pm 406 (20)	1655 \pm 841 (20)	UV-Vis	(55)	
	344 \pm 86 (15)	926 \pm 361 (10)	IC-UV	(222)	Values reported here are for male and female non-smokers combined; standard deviations estimated from relative deviations
	76 (1)	1090 \pm 546 (3)	UV-Vis	(188)	
	77 (1)	1050 \pm 516 (3)	ISE	(188)	
	1050 \pm 350 (3)	2050 \pm 450 (3)	CE	(230)	
	344 \pm 89 (10)	1610 \pm 622 (10)	Electrochemistry	(195)	Also reported ranges for non-smokers (100) and smokers (100) of 200–500 μM and 700–3000 μM , respectively
	612 \pm 133 (6)	2830 \pm 265 (8)	UV-Vis	(193)	“Former smoker” not included in the values reported here
	632 \pm 150 (6)	3000 \pm 356 (8)	FIA	(193)	“Former smoker” not included in the values reported here

(Continued on next page)

TABLE 5
Concentrations of thiocyanate found for smokers vs. nonsmokers (*Continued*)

Biological matrix	Non-smoker (μM)	Smoker (μM)	Analytical method ^a	Reference	Notes ^b
	66–76 (5)	270–4,600 (5)	ISE	(264)	1 non-smoker group and 4 smoker groups; no data given on number of participants in each group; range and not average reported
	1040 \pm 238 (5) 670 \pm 80 (No data)	3620 \pm 1720 (5) 830 \pm 280 (No data)	FIA UV-Vis	(265) (71)	No data given for number of smokers and non-smokers, but authors indicate that the difference was significant
	530 \pm 30 (1)	1350 \pm 40 (1)	ISE	(75)	No data given for number of smokers and non-smokers, but appears to be 1
	560 \pm 30 (1)	1410 \pm 40 (1)	UV-Vis	(75)	No data given for number of smokers and non-smokers, but appears to be 1
	442 \pm 11 (1)	706 \pm 13 (1)	UV-Vis	(74)	No data given for number of smokers and non-smokers, but appears to be 1
	1721 (No data)	3424 \pm 1875 (45)	IC	(66)	No data given for the number of non-smokers, estimated individual SCN concentrations from a figure combining passive, moderate, and heavy smokers
	442 \pm 200 (10)	1958 \pm 230 (30)	UV-Vis	(64)	Only concentration ranges given, combined three test groups for the smokers and averaged
	488 \pm 200 (10)	2425 \pm 283 (60)	ISE	(64)	Only concentration ranges given, combined 6 test groups (both batch and FIA) for the smokers and averaged
	393 \pm 312 (6)	1600 \pm 1150 (5)	IC	(67)	Calculated average and standard deviation from individual data points
	528.4 \pm 30.5 (2) 1400 (1)	3878 \pm 675 (2) 9000 (1)	UV-Vis IC-UV	(81) (216)	2 methods used, both optical

^aSee method notes in Table 3.

^bThe term “plasma” refers to serum or plasma.

ENDOGENOUS CONCENTRATIONS OF CYANIDE AND ITS BREAKDOWN PRODUCTS

When analyzing for cyanide exposure, it is important to note that all biological samples will contain endogenous levels of cyanide (and its biological markers). Therefore, baseline levels of the analyte (cyanide, thiocyanate, ATCA, or cyanide-protein adducts) should be known prior to concluding the occurrence of a cyanide exposure. Table 4 lists reported endogenous concentrations of cyanide in various biological samples from human smokers and non-smokers. Tables 5 and 6 report similar information for thiocyanate and ATCA, respectively (no information

is available for cyanide-protein adducts). The concentrations listed give an idea of background concentrations of cyanide and its breakdown products that may be encountered when determining cyanide exposure. Figures 5–7 illustrate the relationships of cyanide (Figure 5), thiocyanate (Figure 6), and ATCA (Figure 7) concentrations in various biological fluids of smokers and non-smokers (developed from the information reported in Tables 4–6). The figures also contain the intra-study concentration ratios of smokers and non-smokers (e.g., the ratio of the mean cyanide concentration in the urine of smokers to the mean cyanide concentration in the urine of non-smokers in a specific study).

TABLE 6
Concentrations of ATCA found for smokers vs. nonsmokers

Biological matrix	Non-smoker (μM)	Smoker (μM)	Analytical method ^a	Reference	Notes ^b
Blood	0.0808 ± 0.0308 (3)	0.122 ± 0.036 (3)	GC-MS	(61)	Plasma; 27 plasma samples each from 3 smokers and 3 non-smokers
Urine	0.582 ± 0.322 (21)	1.596 ± 1.623 (19)	GC-MS	(91)	Average and standard deviation calculated from individual data reported; data below detection limit not included
	Not detected (10)	0.680 ± 0.332 (4)	HPLC-FLD	(92)	

^aSee method notes in Table 3.

^bThe term "plasma" refers to serum or plasma.

First, it is apparent that for cyanide and thiocyanate, the endogenous concentrations for smokers and non-smokers are inconsistent between studies. This may be due to variations in method robustness, differences in the environment or diet of the study populations used, or difficulties in delayed analysis of cyanide biomarkers addressed above. Inconsistent endogenous concentrations make it difficult to determine cyanide exposure from biological fluids. As seen in Figure 5, blood cyanide concentrations are especially inconsistent, most likely because of differences in storage conditions. For urine cyanide concentrations, most of the smoker concentrations are higher than the

non-smoker concentrations, although there are not enough data to establish the consistency of that relationship. Also, there are not enough data to establish the consistency of cyanide concentrations from saliva. It is important to note that cyanide mainly resides in the red blood cells, so it would be expected that plasma cyanide concentrations reported would be relatively low. Lundquist et al. (133) reported plasma cyanide concentrations of 0.02 and 0.03 μM for smokers and nonsmokers, respectively. These concentrations are two of the lowest in Figure 5, but even if these values were removed, inconsistency in blood cyanide concentrations would remain evident. For thiocyanate,

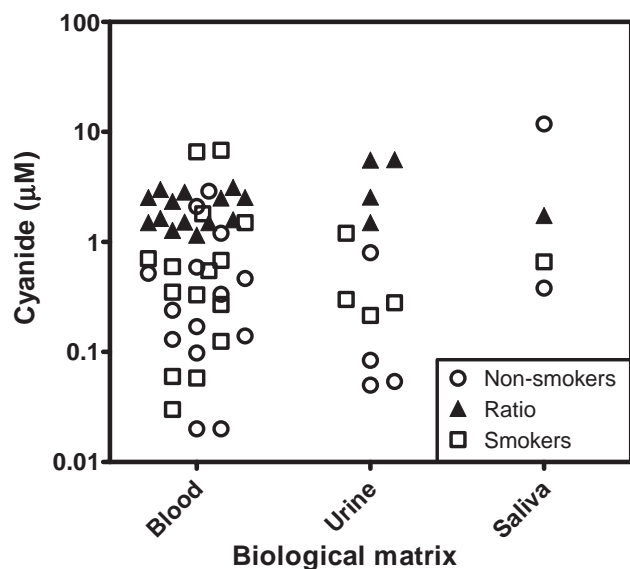


FIG. 5. Compilation of reported cyanide concentrations from biological fluids of human smokers and non-smokers. The ratio illustrated is the intra-study ratio of smoker to non-smoker concentrations of the analyte of interest. The data used to create the figure are reported in Table 4.

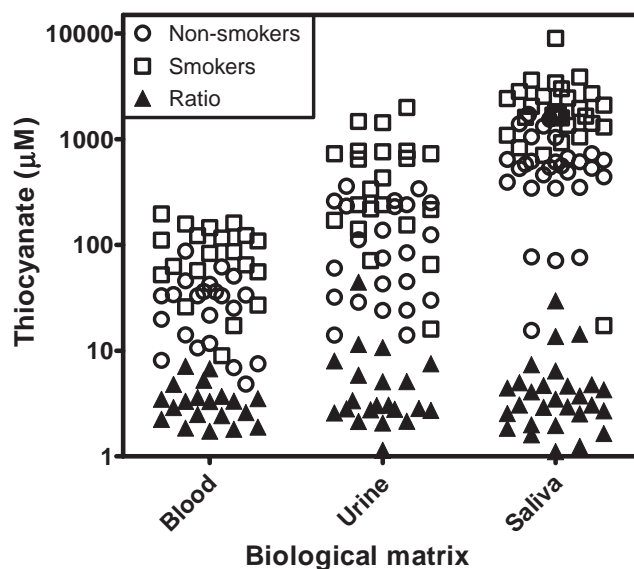


FIG. 6. Compilation of reported thiocyanate concentrations from biological fluids of human smokers and non-smokers. The ratio illustrated is the intra-study ratio of smoker to non-smoker concentrations of the analyte of interest. The data used to create the figure are reported in Table 5.

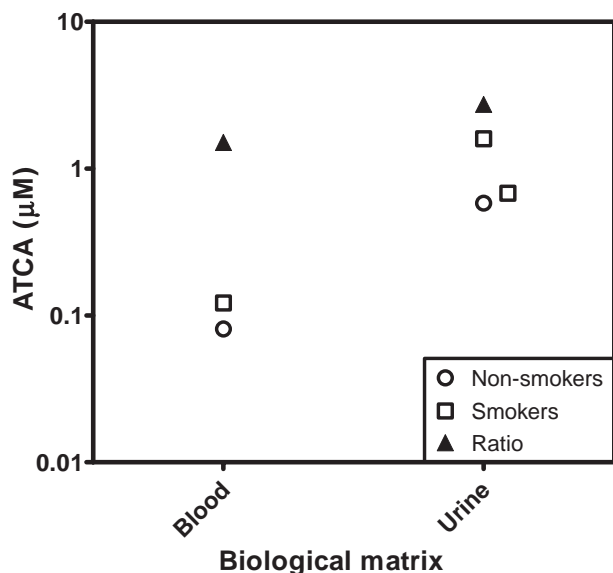


FIG. 7. Compilation of reported ATCA concentrations from biological fluids of human smokers and non-smokers. The ratio illustrated is the intra-study ratio of smoker to non-smoker concentrations of the analyte of interest. The data used to create the figure are reported in Table 6.

there are a large number of studies that report concentrations for each of the biological matrices (Figure 6). From the figure, it appears that plasma offers the best differentiation between thiocyanate concentrations found from smokers and non-smokers. While there is more overlap for both urine and saliva thiocyanate concentrations, the majority of smoker concentrations are above non-smoker concentrations. For ATCA, there is not enough data to derive any conclusions about the consistency of the concentrations in these fluids. Although, for urine, both of the mean concentrations found for ATCA in smokers are higher than those found for non-smokers. It should be noted that the error in urinary analysis of ATCA by GCMS was very large (Table 6) and that a number of individual concentrations for smokers and non-smokers overlapped (91). Therefore, urinary endogenous concentrations of ATCA between studies would be expected to be inconsistent as well. Plasma ATCA analysis of individual smokers and non-smokers was found to be more consistent than urinary analysis (61).

Concentration ratios of smoker to non-smoker are also plotted for cyanide, thiocyanate, and ATCA. While the absolute blood concentrations of cyanide are inconsistent, the intra-study concentration ratios are relatively consistent, ranging from 1.2–3.1. For urine and saliva, these ratios range from 1.5–5.6 for urine and 1.7 for saliva. Overall, these ratios range from 1.2–5.6, and give an indication that the relative ratios of cyanide found in smokers and non-smokers are quite consistent, even if the absolute concentrations are inconsistent. This provides further evidence that the variability in absolute concentrations for cyanide is an artifact of storage conditions (i.e., time and

temperature) and differences in the accuracy of cyanide analysis methods. While the analysis of cyanide concentrations by different methods may contribute to the variability of endogenous concentrations of cyanide reported, Sano et al. (49) found that cyanide concentrations in whole blood for smokers were consistent when comparing IC and fluorescence methods. This example indicates that the major factors contributing to inconsistent cyanide concentrations are associated with storage conditions. For thiocyanate, the intra-study ratios are inconsistent, with ranges of 1.7–7.1, 1.1–44, and 1.1–30 for blood, urine, and saliva, respectively. Each of these ranges is larger than those found for cyanide. This may indicate that thiocyanate is produced or depleted by processes other than cyanide exposure. For ATCA, there is limited data, but it appears that the intra-study ratios (1.5 for plasma and 2.7 for urine) are in good agreement with the ratios found for cyanide. This may indicate that ATCA is produced mainly from cyanide exposure and little is used or produced in other biological processes.

CONCLUSIONS

The analytical determination of biological markers of cyanide exposure is not an easy task due to chemical properties, biological activities, and limited published research (for certain markers of cyanide exposure). Numerous methods have been developed and each has its own advantages and disadvantages. However, they have all provided insight into the verification of cyanide exposure from analysis of biological samples. Table 3 provides a comparison of analytical techniques for analysis of cyanide or its metabolites based on sensitivity, specificity, sample size, capacity, expertise necessary to perform the method, and cost. Some other key pieces of information should also be considered prior to choosing a method to perform:

1. Was preservation of cyanide and its metabolites during storage addressed?
2. Were typical interferences for the biological matrix of interest removed?
3. Were analysis procedures that could result in the loss of cyanide or its metabolites used (i.e., heating or acidification)?

For the analysis of cyanide, the largest inconsistency in the literature is analysis with different preservation techniques. For cyanide analysis, biological samples should be collected and analyzed as soon as possible and regardless of the analytical method, a preserving technique needs to be considered so that accurate concentrations of cyanide can be found (130, 133, 150, 181, 254–258). It should also be considered that all biological samples will contain endogenous levels of cyanide (and its biological markers). Therefore, baseline levels of the analyte measured should be known prior to concluding occurrence of a cyanide exposure. Problems with direct analysis of cyanide, including short half-lives, artifactual formation of cyanide, and interconversion of cyanide and thiocyanate, contribute to difficulties in the analysis of cyanide for all analytical methods designed to determine cyanide in biological samples.

While cyanide is most often analyzed to determine cyanide exposure, analytical techniques that analyze for breakdown products of cyanide as well as those that analyze for cyanide directly should be considered. Although cyanide metabolites may offer longer half-lives, they also have a number of drawbacks. For thiocyanate, the main drawback is large and variable background concentrations in biological samples. Other disadvantages include the conversion of cyanide and thiocyanate and the use of thiocyanate by other biological processes not directly related to cyanide metabolism. For ATCA and cyanide-protein adducts, the main drawback is the limited amount of research available on toxicokinetics and relationships of these metabolites to cyanide exposure. Care should be taken when choosing an analytical method to consider not just the parameters of the analytical method but also the toxicokinetics of cyanide and its metabolites.

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REFERENCES

1. A. P. Knight and R. G. Walter, Plants causing sudden death, in *A Guide to Plant Poisoning of Animals in North America* eds. A. P. Knight and R. G. Walter, 1–56 (Teton NewMedia, Jackson, WY, 2002).
2. B. C. McGorum and R. A. Anderson, Biomarkers of exposure to cyanogens in horses with grass sickness. *The Veterinary Record* 151 (2002):442–445.
3. J. Vetter, Plant cyanogenic glycosides. *Toxicon* 38 (2000):11–36.
4. D. H. Honig, M. E. Hockridge, R. M. Gould, and J. J. Rackis, Determination of cyanide in soybeans and soybean products. *Journal of Agricultural and Food Chemistry* 31 (1983):272–275.
5. F. Nartey, Toxicological aspects of cyanogenesis in tropical food-stuffs, in *Toxicology in the Tropics* eds. R. L. Smith and E. A. Bababummi (Taylor & Francis Ltd. London, 1980), 53–73.
6. E. E. Conn, Cyanogenic glycosides. *International Review of Biochemistry* 27 (1979):21–43.
7. C. M. Hibbs, Cyanide and nitrate toxicoses of cattle. *Veterinary and Human Toxicology* 21 (1979):401–403.
8. Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological Profile for Cyanide (U.S. Dept. of Health and Human Services, Atlanta, GA, 2004).
9. Q. Li, D. J. Jacob, I. Bey, R. M. Yantosca, Y. Zhao, Y. Kondo, and J. Notholt, Atmospheric hydrogen cyanide (HCN): Biomass burning source, ocean sink? *Geophysical Research Letters* 27 (2000):357–360.
10. J. L. Way, Cyanide intoxication and its mechanism of antagonism. *Annual Review of Pharmacology and Toxicology* 24 (1984):451–481.
11. L. P. Solomonson, Cyanide as a metabolic inhibitor, in *Cyanide in Biology* eds. B. Vennesland, E. E. Conn, C. J. Knowles, J. Westley, and F. Wissing (Academic Press, New York, 1981), 11–28.
12. L. E. Towill, J. S. Drury, B. L. Whitfield, E. B. Lewis, E. L. Galyan, and A. S. Hammons, *Reviews of the Environmental Effects of Pollutants: Part V, Cyanide* (EPA 600/1-78-027, Office of Research and Development, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH, 1978).
13. J. L. Way, Pharmacologic aspects of cyanide and its antagonism, in *Cyanide in Biology* eds. B. Vennesland, E. E. Conn, C. J. Knowles, J. Westley, and F. Wissing (Academic Press, New York, 1981), 29–49.
14. World Health Organization, Cyanide in drinking water: Background document for development of WHO guidelines for drinking water quality (WHO/SDE/WSH/03.04/05, World Health Organization, Geneva, 2007).
15. R. Schnepf, Cyanide: Sources, perceptions, and risks. *Journal of Emergency Nursing* 32 (2006):S3–S7.
16. K. A. Wolnik, F. L. Fricke, E. Bonnin, C. M. Gaston, and R. D. Satzger, The Tylenol tampering incident—Tracing the source. *Analytical Chemistry* 56 (1984):466A–470A, 474A.
17. M. Eckstein, Cyanide as a chemical terrorism weapon. *Journal of Emergency Medical Services* 29 (2004):22–31.
18. K. K. W. Mak, H. Yanase, and R. Renneberg, Cyanide fishing and cyanide detection in coral reef fish using chemical tests and biosensors. *Biosensors & Bioelectronics* 20 (2005):2581–2593.
19. P. J. Rubec, The effect of sodium cyanide on coral reefs and marine fish in the Philippines, in *The First Asian Fish Forum* eds. J. L. Maclean, L. B. Dizon, and L. V. Hosillos (Asian Fisheries Society, Manila, Philippines, 1986), 297–302.
20. C. V. Barber and V. R. Pratt, Poison and profits: Cyanide fishing in the Indo-Pacific. *Environment* 40 (1998):4–9, 28–34.
21. S. I. Baskin, I. Petrikovics, J. S. Kurche, J. D. Nicholson, B. A. Logue, B. J. Maliner, and G. A. Rockwood, Insights on cyanide toxicity and methods of treatment, in *Pharmacological Perspectives of Toxic Chemicals and Their Antidotes* eds. S. J. S. Flora, J. A. Romano, Jr., S. I. Baskin, and K. Sekhar (Narosa Publishing House, New Delhi, 2004), Ch. 9, 105–146.
22. A. E. Lindsay, A. R. Greenbaum, and D. O'Hare, Analytical techniques for cyanide in blood and published blood cyanide concentrations from healthy subjects and fire victims. *Analytica Chimica Acta* 511 (2004):185–195.
23. M. G. Valdes and M. E. Diaz-Garcia, Determination of thiocyanate within physiological fluids and environmental samples: Current practice and future trends. *Critical Reviews in Analytical Chemistry* 34 (2004):9–23.
24. C. M. Troup and B. Ballantyne, Analysis of cyanide in biological fluids and tissues, in *Clinical and Experimental Toxicology of Cyanides* eds. B. Ballantyne and T. C. Marrs (Wright Publishers, Bristol, 1987), 22–40.
25. L. S. Bark and H. G. Higson, A review of the methods available for the detection and determination of small amounts of cyanide. *Analyst* 88 (1963):751–760.

26. D. J. Barillo, Diagnosis and treatment of cyanide toxicity. *Journal of Burn Care & Research* 30 (2009):148–152.
27. D. E. Metzler, *Biochemistry: The Chemical Reactions of Living Cells*, 2nd ed. (Academic Press, San Diego, 2001).
28. G. E. Isom and S. I. Baskin, Enzymes involved in cyanide metabolism, in *Comprehensive Toxicology* eds. I. G. Sipes, C.A. McQueen, and A. J. Gandolfi (Elsevier Science, New York, 1997), 477–488.
29. A. Salkowski and D. Penney, Cyanide poisoning in animals and humans: A review. *Veterinary and Human Toxicology* 36 (1994):455–466.
30. A. B. Sousa, H. Manzano, B. Soto-Blanco, and S. L. Górniak, Toxicokinetics of cyanide in rats, pigs and goats after oral dosing with potassium cyanide. *Archives of Toxicology* 77 (2004):330–334.
31. J. L. Wood and S. L. Cooley, Detoxication of cyanide by cystine. *Journal of Biological Chemistry* 218 (1956):449–457.
32. J. L. Borowitz, G. E. Isom, and S. I. Baskin, Acute and chronic cyanide toxicity, in *Chemical Warfare Agents: Toxicity at Low Levels* eds. S. M. Somani and J. A. Romano, Jr. (CRC Press, Boca Raton, 2001), 301–319.
33. S. I. Baskin and T. G. Brewer, Cyanide poisoning, in *Textbook of Military Medicine, Medical Aspects of Chemical and Biological Warfare* eds. F. R. Sidell, E. T. Takafuji, and D. R. Franz (Borden Institute, Washington, DC, 1997), Ch. 10, 271–286.
34. M. J. Fasco, C. R. Hauer III, R. F. Stack, C. O'Hehir, J. R. Barr, and G. A. Eadon, Cyanide adducts with human plasma proteins: Albumin as a potential exposure surrogate. *Chemical Research in Toxicology* 20 (2007):677–684.
35. D. Noort, H. P. Benschop, and R. M. Black, Biomonitoring of exposure to chemical warfare agents: A review. *Toxicology and Applied Pharmacology* 184 (2002):116–126.
36. P. C. Turner, K. H. Dingley, J. Coxhead, S. Russell, and C. R. Garner, Detectable levels of serum aflatoxin B1-albumin adducts in the United Kingdom population: Implications for aflatoxin-B1 exposure in the United Kingdom. *Cancer Epidemiology, Biomarkers and Prevention* 7 (1998):441–447.
37. L. Dirikolu, C. Hughes, D. Harkins, J. Boyles, J. Bosken, F. Lehner, A. Troppmann, K. McDowell, T. Tobin, M. Sebastian, L. Harrison, J. Crutchfield, S. I. Baskin, and T. D. Fitzgerald, The toxicokinetics of cyanide and mandelonitrile in the horse and their relevance to the mare reproductive loss syndrome. *Toxicology Mechanisms and Methods* 13 (2003):199–211.
38. R. P. Lanno and D. G. Dixon, The comparative chronic toxicity of thiocyanate and cyanide to rainbow trout (*Oncorhynchus mykiss*). *Aquatic Toxicology* 36 (1996):177–187.
39. R. P. Lanno and D. G. Dixon, Chronic toxicity of waterborne thiocyanate to rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences* 53 (1996):2137–2146.
40. D. G. Brown, R. P. Lanno, M. R. van den Heuvel, and D. G. Dixon, HPLC determination of plasma thiocyanate concentrations in fish blood: Application to laboratory pharmacokinetic and field-monitoring studies. *Ecotoxicology and Environmental Safety* 30 (1995):302–308.
41. R. P. Lanno and D. G. Dixon, Chronic toxicity of waterborne thiocyanate to the fathead minnow (*Pimephales promelas*). *Environmental Toxicology and Chemistry* 13 (1993):1423–1432.
42. R. Garcia, Cyanide, in *Medical Response to Terrorism* eds. D. C. Keyes, J. L. Burstein, R. B. Schwartz, and R. E. Sweinton (Lippincott Williams and Wilkins, Philadelphia, 2004), Ch. 3, 26–37.
43. N. P. Okolie and A. U. Osagie, Liver and kidney lesions and associated enzyme changes induced in rabbits by chronic cyanide exposure. *Food and Chemical Toxicology* 37 (1999):745–750.
44. I. Swenne, U. J. Eriksson, R. Christoffersson, B. Kågedal, P. Lundquist, L. Nilsson, T. Tylleskär, and H. Rosling, Cyanide detoxification in rats exposed to acetonitrile and fed a low protein diet. *Toxicological Sciences* 32 (1996):66–71.
45. A. M. Calafat and S. B. Stanfill, Rapid quantitation of cyanide in whole blood by automated headspace gas chromatography. *Journal of Chromatography B* 772 (2002):131–137.
46. R. Hartung, Cyanides and nitriles, in *Patty's Industrial Hygiene and Toxicology*, Vol. 2C eds. G. D. Clayton and E. Clayton (John Wiley & Sons, New York, NY, 1982), 4845–4900.
47. F. Moriya and Y. Hashimoto, Potential for error when assessing blood cyanide concentrations in fire victims. *Journal of Forensic Sciences* 46 (2001):1421–1425.
48. F. L. Rodkey and R. F. Robertson, Analytical precautions in measurement of blood cyanide. *Clinical Chemistry* 24 (1978):2184–2185.
49. A. Sano, M. Takezawa, and S. Takitani, Spectrofluorometric determination of cyanide in blood and urine with naphthalene-2,3-dialdehyde and taurine. *Analytica Chimica Acta* 225 (1989):351–358.
50. A. Sano, M. Takezawa, and S. Takitani, High performance liquid chromatography determination of cyanide in urine by pre-column fluorescence derivatization. *Biomedical Chromatography* 3 (1989):209–212.
51. O. Suzuki, H. Hattori, M. Oya, and Y. Katsumata, Direct fluorometric determination of cyanide in human materials. *Forensic Science International* 19 (1982):189–195.
52. T. Imanari, S. Tanabe, and T. Toida, Simultaneous determination of cyanide and thiocyanate by high-performance liquid chromatography. *Chemical & Pharmaceutical Bulletin* 30 (1982):3800–3802.
53. K. Funazo, M. Tanaka, and T. Shono, Determination of cyanide or thiocyanate at trace levels by derivatization and gas chromatography with flame thermionic detection. *Analytical Chemistry* 53 (1981):1377–1380.
54. B. D. Paul and M. L. Smith, Cyanide and thiocyanate in human saliva by gas chromatography-mass spectrometry. *Journal of Analytical Toxicology* 30 (2006):511–515.
55. K. Tsuge, M. Kataoka, and Y. Seto, Cyanide and thiocyanate levels in blood and saliva of healthy adult volunteers. *Journal of Health Science* 46 (2000):343–350.
56. K. Stamyr, P. Nord, and G. Johanson, Washout kinetics of inhaled hydrogen cyanide in breath. *Toxicology Letters* 179 (2008):59–62.
57. J. Raju and V. K. Gupta, Spectrophotometric determination of hydrogen cyanide in air and its application in biological samples. *Asian Environment* 11 (1989):66–72.
58. P. Lundquist, H. Rosling, and B. Sörbo, The origin of hydrogen cyanide in breath. *Archives of Toxicology* 61 (1988):270–274.
59. P. Kaur, S. Upadhyay, and V. K. Gupta, Spectrophotometric determination of hydrogen cyanide in air and biological fluids. *Analyst* 112 (1987):1681–1683.

60. G. E. Boxer and J. C. Rickards, Determination of traces of hydrogen cyanide in respiratory air. *Archives of Biochemistry and Biophysics* 39 (1952):287–291.
61. B. A. Logue, W. K. Maserek, G. A. Rockwood, M. W. Keebaugh, and S. I. Baskin, The analysis of 2-amino-2-thiazoline-4-carboxylic acid in the plasma of smokers and non-smokers. *Toxicology Mechanisms and Methods* 19 (2009):202–208.
62. S. Ershad, L. A. Sagathforoush, and G. Karim-Nezhad, A selective optical chemosensor based on a thia-containing Schiff-base iron(III) complex for thiocyanate ion. *Analytical Sciences* 25 (2009):665–668.
63. B. Patel, A. Kumar, and S. K. Menon, Thiocyanate: selective membrane electrode based on macrotricyclic binuclear Cu(II)-Schiff base complex. *Journal of Inclusion Phenomena and Macrocyclic Chemistry* 64 (2009):239–247.
64. S. S. M. Hassan, I. H. A. Badr, A. H. Kamel, and M. S. Mohamed, A novel poly(vinyl chloride) matrix membrane sensor for batch and flow-injection determinations of thiocyanate, cyanide and some metal ions. *Analytical Sciences* 25 (2009):911–917.
65. Y. Y. Dong, C. Y. Li, X. B. Zhang, R. Q. Yu, and G. L. Shen, A novel potentiometric sensor for thiocyanate based on an amide-linked manganese diporphyrin xanthene. *Electroanalysis* 20 (2008):1769–1774.
66. I. Demkowska, Z. Polkowska, and J. Namiesnik, Application of ion chromatography for the determination of inorganic ions, especially thiocyanates in human saliva samples as biomarkers of environmental tobacco smoke exposure. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* 875 (2008):419–426.
67. M. Mori, T. Iwata, T. Satori, S. I. Ohira, H. Itabashi, and K. Tanaka, Ion-exclusion/cation-exchange chromatographic determination of common inorganic ions in human saliva by using an eluent containing zwitterionic surfactant. *Journal of Chromatography A* 1213 (2008):125–129.
68. M. Mazloun-Ardakani, M. A. S. Mohseni, and M. Salavati-Niasari, Novel thiocyanate-selective electrode based on binuclear molybdenum complex of bis-N,O-bidentate Schiff base. *Canadian Journal of Analytical Sciences and Spectroscopy* 53 (2008):179–188.
69. A. Shokrollahi, M. Ghaedi, H. Ghaedi, and A. H. Kianfar, Thiocyanate-selective membrane electrode based on cobalt(III) Schiff base as a charge carrier. *International Journal of Environmental Analytical Chemistry* 88 (2008):841–856.
70. Z. Q. Xu, T. Doi, A. R. Timerbaev, and T. Hirokawa, Sensitive determination of anions in saliva using capillary electrophoresis after transient isotachophoretic preconcentration. *Talanta* 77 (2008):278–281.
71. L. Minarowski, D. Sands, A. Minarowska, A. Karwowska, A. Sulewska, M. Gacko, and E. Chyczewska, Thiocyanate concentration in saliva of cystic fibrosis patients. *Folia Histochemica Et Cytobiologica* 46 (2008):245–246.
72. E. Saussereau, J. P. Goulle, and C. Lacroix, Determination of thiocyanate in plasma by ion chromatography and ultraviolet detection. *Journal of Analytical Toxicology* 31 (2007):383–387.
73. M. M. Ardakani, M. A. Karimi, R. Mazidi, H. Naeimi, and K. Rabiei, A selective membrane electrode for thiocyanate ion based on N,N'-1,1-isobutanebis-(salicylaldimidinato) uranyl (II). *Canadian Journal of Analytical Sciences and Spectroscopy* 52 (2007):233–242.
74. S. Rastegarzadeh and Z. Moradpour, A novel optical sensor for determination of thiocyanate. *Analytical Letters* 40 (2007):2993–3001.
75. P. H. Yang, W. Z. Wei, and C. Y. Tao, Determination of trace thiocyanate with nano-silver coated multi-walled carbon nanotubes modified glassy carbon electrode. *Analytica Chimica Acta* 585 (2007):331–336.
76. K. V. Yablotskiy, O. V. Radhul, I. A. Veselova, and T. N. Shekhovtsova, Determination of fluoride, cyanide, and thiocyanate using horseradish peroxidase immobilized on modified silica gel. *Analytical Letters* 40 (2007):1521–1539.
77. N. Goi, K. Takagi, Y. Hirai, H. Harada, A. Kari, Y. Terashima, N. Kinae, M. Hiramatsu, K. Nakamura, and T. Ono, Effect of psychologic stress on peroxidase and thiocyanate levels in human saliva detected by ultraweak chemiluminescence. *Journal of Health Science* 53 (2007):161–169.
78. A. S. Bashammakh, S. O. Bahaffi, A. A. Al-Sibaa, H. O. Al-Wael, and M. S. El-Shahawi, Extractive liquid-liquid spectrophotometric procedure for the determination of thiocyanate ions employing the ion pair reagent amiloride monohydrochloride. *Analytica Chimica Acta* 592 (2007):16–23.
79. F. C. Wang, Y. Q. Chai, and R. Yuan, Thiocyanate-selective electrode based on N-salicylidene-benzylamineato copper(II) complex. *Russian Journal of Electrochemistry* 44 (2008):272–277.
80. M. M. Ardakani, M. Jamshidpour, H. Naeimi, and L. Moradi, Thiocyanate ion-selective PVC membrane electrode based on N,N'-ethylene-bis(4-methylsalicylidineiminato)nickel(II). *Analytical Sciences* 22 (2006):1221–1226.
81. M. S. Garcia, J. A. Ortuno, C. Sanchez-Pedreno, M. I. Alberro, and M. J. Fernandez, Flow-through bulk optode for spectrophotometric determination of thiocyanate and its application to water and saliva analysis. *Sensors* 6 (2006):1224–1233.
82. A. R. Pettigrew and G. S. Fell, Microdiffusion method for estimation of cyanide in whole blood and its application to the study of conversion of cyanide to thiocyanate. *Clinical Chemistry* 19 (1973):466–471.
83. K. Diem and C. Lentner, *Geigy Scientific Tables*, 7th ed. (Geigy Pharmaceuticals, Ardsley, NY, 1975).
84. S. Yamanaka, S. Takaku, Y. Takaesu, and M. Nishimura, Validity of salivary thiocyanate as an indicator of cyanide exposure from smoking. *Bulletin of Tokyo Dental College* 32 (1991):157–163.
85. M. J. Jarvis, Application of biochemical intake markers to passive smoking measurement and risk estimation. *Mutation Research* 222 (1989):101–110.
86. T. F. Maliszewski and B. D.E., True and apparent thiocyanate in body fluids of smokers and nonsmokers. *Journal of applied Physiology* 8 (1955):289–291.
87. J. Pre and R. Vassy, Plasma thiocyanate and cigarette-smoking status. *Medical Science Research* 20 (1992):2.
88. Y. Hasuike, T. Nakanishi, R. Moriguchi, Y. Otaki, M. Nanami, Y. Hama, M. Naka, K. Miyagawa, M. Izumi, and Y. Takamitsu, Accumulation of cyanide and thiocyanate in haemodialysis patients. *Nephrology Dialysis Transplantation* 19 (2004):1474–1479.
89. L. S. Bradham, N. Catsimpoolas, and J. L. Wood, Determination of 2-iminothiazolidine-4-carboxylic acid. *Analytical Biochemistry* 11 (1965):230–232, discussion 232–237.

90. S. I. Baskin, I. Petrikovics, G. E. Platoff, G. A. Rockwood, and B. A. Logue, Spectrophotometric analysis of the cyanide metabolite 2-aminothiazoline-4-carboxylic acid (ATCA). *Toxicology Mechanisms and Methods* 16 (2006):339–345.
91. B. A. Logue, N. P. Kirschten, I. Petrikovics, M. A. Moser, G. A. Rockwood, and S. I. Baskin, Determination of the cyanide metabolite 2-aminothiazoline-4-carboxylic acid in urine and plasma by gas chromatography-mass spectrometry. *Journal of Chromatography B* 819 (2005):237–244.
92. P. Lundquist, B. Kågedal, L. Nilsson, and H. Rosling, Analysis of the cyanide metabolite 2-aminothiazoline-4-carboxylic acid in urine by high-performance liquid chromatography. *Analytical Biochemistry* 228 (1995):27–34.
93. A. R. Pettigrew and G. S. Fell, Simplified colorimetric determination of thiocyanate in biological fluids, and its application to investigation of the toxic amblyopias. *Clinical Chemistry* 18 (1972):996–1000.
94. X. Liu and Z. Yun, High-performance liquid chromatographic determination of thiocyanate anion by derivatization with pentafluorobenzyl bromide. *Journal of Chromatography* 653 (1993):348–353.
95. C. Maseda, K. Matsubara, and H. Shiono, Improved gas chromatography with electron-capture detection using a reaction pre-column for the determination of blood cyanide: A higher content in the left ventricle of fire victims. *Journal of Chromatography* 490 (1989):319–327.
96. R. H. Cardozo and I. S. Edelman, The volume of distribution of sodium thiosulfate as a measure of the extracellular fluid space. *The Journal of Clinical Investigation* 31 (1952):280–290.
97. B. Ballantyne, Factors in the analysis of whole blood thiocyanate. *Clinical Toxicology* 11 (1977):195–210.
98. J. L. Wood, E. F. J. Williams, and N. Kingsland, The conversion of thiocyanate sulfur to sulfate in the white rat. *Journal of Biological Chemistry* 170 (1947):251–259.
99. J. L. Wood and E. F. J. Williams, The metabolism of thiocyanate in the rat and its inhibition by propylthiouracil. *Journal of Biological Chemistry* 177 (1949):59–67.
100. W. Weuffen, G. Jess, W. D. Julich, and D. Bernhart, Studies on the relationship between 2-iminothiazolidine-4-carboxylic acid and the thiocyanate metabolism in the guinea-pig. *Pharmazie* 35 (1980):221–223.
101. R. S. Bitner, G. K. Yim, and G. E. Isom, 2-Iminothiazolidine-4-carboxylic acid produces hippocampal CA1 lesions independent of seizure excitation and glutamate receptor activation. *Neurotoxicology* 18 (1997):191–200.
102. R. S. Bitner, A. Kanthasamy, G. E. Isom, and G. K. Yim, Seizures and selective CA-1 hippocampal lesions induced by an excitotoxic cyanide metabolite, 2-iminothiazolidine-4-carboxylic acid. *Neurotoxicology* 16 (1995):115–122.
103. S. Felby, Determination of cyanide in blood by reaction head-space gas chromatography. *Forensic Sci. Med. Pathol.* 5 (2009):39–43.
104. N. Sadeg and H. Belhadj-Tahar, Rapid and sensitive headspace gas chromatographic method for cyanide determination in whole blood. *Toxicological and Environmental Chemistry* 91 (2009):419–424.
105. V. Gambaro, S. Arnoldi, E. Casagni, L. Dell'Acqua, C. Pecoraro, and R. Froldi, Blood cyanide determination in two cases of fatal intoxication: Comparison between headspace gas chromatography and a spectrophotometric method. *Journal of Forensic Sciences* 52 (2007):1401–1404.
106. A. E. Lindsay and D. O'Hare, The development of an electrochemical sensor for the determination of cyanide in physiological solutions. *Analytica Chimica Acta* 558 (2006):158–163.
107. G. Frison, F. Zancanaro, D. Favretto, and S. D. Ferrara, An improved method for cyanide determination in blood using solid-phase microextraction and gas chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry* 20 (2006):2932–2938.
108. P. Dumas, G. Gingras, and A. LeBlanc, Isotope dilution-mass spectrometry determination of blood cyanide by headspace gas chromatography. *Journal of Analytical Toxicology* 29 (2005):71–75.
109. J. Lv, Z. Zhang, J. Li, and L. Luo, A micro-chemiluminescence determination of cyanide in whole blood. *Forensic Science International* 148 (2005):15–19.
110. A. Tracqui, J. S. Raul, A. Gerault, L. Berthelon, and B. Ludes, Determination of blood cyanide by HPLC-MS. *Journal of Analytical Toxicology* 26 (2002):144–148.
111. H. Meiser, H.-W. Hagedorn, and R. Schulz, Development of a method for determination of cyanide concentrations in serum and rumen fluid of cattle. *American Journal of Veterinary Research* 61 (2000):658–664.
112. S. Chinaka, N. Takayama, Y. Michigami, and K. Ueda, Simultaneous determination of cyanide and thiocyanate in blood by ion chromatography with fluorescence and ultraviolet detection. *Journal of Chromatography B* 713 (1998):353–359.
113. K. Takekawa, M. Oya, A. Kido, and O. Suzuki, Analysis of cyanide in blood by headspace solid-phase microextraction (SPME) and capillary gas chromatography. *Chromatographia* 47 (1998):209–214.
114. A. Ishii, H. Seno, K. Watanabe-Suzuki, O. Suzuki, and T. Kumazawa, Determination of cyanide in whole blood by capillary gas chromatography with cryogenic oven trapping. *Analytical Chemistry* 70 (1998):4873–4876.
115. D. Felscher and M. Wulfmeyer, A new specific method to detect cyanide in body fluids, especially whole blood, by fluorimetry. *Journal of Analytical Toxicology* 22 (1998):363–366.
116. F. J. Baud, S. W. Borron, E. Bavoux, A. Astier, and J. R. Hoffman, Relation between plasma lactate and blood cyanide concentrations in acute cyanide poisoning. *British Medical Journal (Clinical Research)* 312 (1996):26–27.
117. S. Kage, T. Nagata, and K. Kudo, Determination of cyanide and thiocyanate in blood by gas chromatography and gas chromatography-mass spectrometry. *Journal of Chromatography B* 675 (1996):27–32.
118. S. Kage, T. Nagata, and K. Kudo, Determination of cyanide in blood by GC-ECD and GC-MS. *Japanese Journal of Forensic Toxicology* 12 (1994):130–131.
119. M. Odoul, B. Fouillet, B. Nouri, R. Chambon, and P. Chambon, Specific determination of cyanide in blood by headspace gas chromatography. *Journal of Analytical Toxicology* 18 (1994):205–207.
120. M. Laforge, F. Buneaux, P. Houeto, F. Bourgeois, R. Bourdon, and P. Levillain, A rapid spectrophotometric blood cyanide determination applicable to emergency toxicology. *Journal of Analytical Toxicology* 18 (1994):173–175.

121. Y. Seto, N. Tsunoda, H. Ohta, and T. Shinohara, Determination of blood cyanide by headspace gas chromatography with nitrogen-phosphorus detection and using a megabore capillary column. *Analytica Chimica Acta* 276 (1993):247–259.
122. A. Sano, N. Takimoto, and S. Takitani, High-performance liquid chromatographic determination of cyanide in human red blood cells by pre-column fluorescence derivatization. *Journal of Chromatography* 582 (1992):131–135.
123. F. J. Baud, P. Barriot, V. Toffis, B. Riou, E. Vicaud, Y. Lecarpentier, R. Bourdon, A. Astier, and C. Bismuth, Elevated blood cyanide concentrations in victims of smoke inhalation. *The New England Journal of Medicine* 325 (1991):1761–1766.
124. H. Shiono, C. Maseda, A. Akane, and K. Matsubara, The blood cyanide level in fire victims. Rapid and sensitive quantitation of cyanide in blood and its application to autopsy cases. *Hanzaigaku Zasshi* 57 (1991):73–79.
125. H. Shiono, C. Maseda, A. Akane, and K. Matsubara, Rapid and sensitive quantitation of cyanide in blood and its application to fire victims. *The American Journal of Forensic Medicine and Pathology* 12 (1991):50–53.
126. B. C. Levin, P. R. Rechani, J. L. Gurman, F. Landron, H. M. Clark, M. F. Yoklavich, J. R. Rodriguez, L. Droz, F. Mattos de Cabrera, and S. Kaye, Analysis of carboxyhemoglobin and cyanide in blood from victims of the Dupont Plaza Hotel fire in Puerto Rico. *Journal of Forensic Sciences* 35 (1990):151–168.
127. Y. Seto, T. Shinohara, and N. Tsunoda, Head-space capillary gas chromatography of cyanide in blood. *Japanese Journal of Forensic Toxicology* 8 (1990):56–57.
128. C. Maseda, K. Matsubara, M. Hasegawa, A. Akane, and H. Shiono, Determination of blood cyanide using head-space gas chromatography with electron capture detection. *Nippon Hoigaku Zasshi* 44 (1990):131–136.
129. A. M. Westley and J. Westley, Voltammetric determination of cyanide and thiocyanate in small biological samples. *Analytical Biochemistry* 181 (1989):190–194.
130. P. Lundquist and B. Sörbo, Rapid determination of toxic cyanide concentrations in blood. *Clinical Chemistry* 35 (1989):617–619.
131. J. Zamecnik and J. Tam, Cyanide in blood by gas chromatography with NP detector and acetonitrile as internal standard. Application on air accident fire victims. *Journal of Analytical Toxicology* 11 (1987):47–48.
132. P. Lundquist, H. Rosling, B. Sörbo, and L. Tibbling, Cyanide concentrations in blood after cigarette smoking, as determined by a sensitive fluorometric method. *Clinical Chemistry* 33 (1987):1228–1230.
133. P. Lundquist, H. Rosling, and B. Sörbo, Determination of cyanide in whole blood, erythrocytes, and plasma. *Clinical Chemistry* 31 (1985):591–595.
134. T. Toida, T. Togawa, S. Tanabe, and T. Imanari, Determination of cyanide and thiocyanate in blood plasma and red cells by high-performance liquid chromatography with fluorometric detection. *Journal of Chromatography* 308 (1984):133–141.
135. F. McAuley and D. S. Reive, Rapid quantitation of cyanide in blood by gas chromatography. *Journal of Analytical Toxicology* 7 (1983):213–215.
136. C. J. Clark, D. Campbell, and W. H. Reid, Blood carboxy-hemoglobin and cyanide levels in fire survivors. *Lancet* 1 (1981):1332–1335.
137. R. W. Darr, T. L. Capson, and F. D. Hileman, Determination of hydrogen cyanide in blood using gas chromatography with alkali thermionic detection. *Analytical Chemistry* 52 (1980):1379–1381.
138. B. H. McAnalley, W. T. Lowry, R. D. Oliver, and J. C. Garriott, Determination of inorganic sulfide and cyanide in blood using specific ion electrodes: Application to the investigation of hydrogen sulfide and cyanide poisoning. *Journal of Analytical Toxicology* 3 (1979):111–114.
139. F. L. Rodkey and H. A. Collison, Determination of cyanide and nitroprusside in blood and plasma. *Clinical Chemistry* 23 (1977):1969–1975.
140. B. Ballantyne, Blood, brain, and cerebrospinal fluid cyanide concentrations in experimental acute cyanide poisoning. *Journal of the Forensic Science Society* 15 (1975):51–56.
141. S. Baar, The micro determination of cyanide: Its application to the analysis of whole blood. *Analyst* 91 (1966):268–272.
142. A. Sano, M. Takezawa, and S. Takitani, High performance liquid chromatography determination of cyanide in urine by pre-column fluorescence derivatization. *Biomedical Chromatography* 3 (2005):209–212.
143. A. Tomoda and K. Hashimoto, The determination of cyanide in water and biological tissues by methemoglobin. *Journal of Hazardous Materials* 28 (1991):241–249.
144. M. Bogusz, Disappearance of cyanide in blood and tissues after fatal poisoning. *Forensic Science* 7 (1976):173.
145. M. Ghaedi, H. Parham, M. Moghadam, K. Mortazavi, and M. Soylak, Solid phase extraction and spectrophotometric determination of trace amounts of thiocyanate in real samples. *Annali Di Chimica* 96 (2006):689–696.
146. K. W. Mak, H. Yanase, and R. Renneberg, Novel optical biotest for determination of cyanide traces in marine fish using microbial cyanide hydratase and formate dehydrogenase. *Microchimica Acta* 149 (2005):131–135.
147. C. Hughes, F. Lehner, L. Dirikolu, D. Harkins, J. Boyles, K. McDowell, T. Tobin, J. Crutchfield, M. Sebastian, L. Harrison, and S. I. Baskin, A simple and highly sensitive spectrophotometric method for the determination of cyanide in equine blood. *Toxicology Mechanisms and Methods* 13 (2003):129–138.
148. M. R. Haque and J. H. Bradbury, Simple method for determination of thiocyanate in urine. *Clinical Chemistry* 45 (1999):1459–1464.
149. M. Lahti, J. Vilpo, and J. Hovinen, Spectrophotometric determination of thiocyanate in human saliva. *Journal of Chemical Education* 76 (1999):1281–1282.
150. C. J. Vesey, H. McAllister, and R. M. Langford, A simple, rapid and sensitive semimicro method for the measurement of cyanide in blood. *Annals of Clinical Biochemistry* 36 (1999):755–758.
151. C. J. Vesey, H. McAllister, and R. M. Langford, A safer method for the measurement of plasma thiocyanate. *Journal of Analytical Toxicology* 23 (1999):134–136.
152. Z. L. Cardeal, J. P. Gallet, A. Astier, and D. Pradeau, Cyanide assay: Statistical comparison of a new gas chromatographic calibration method versus the classical spectrophotometric method. *Journal of Analytical Toxicology* 19 (1995):31–34.
153. P. Lundquist, B. Kågedal, and L. Nilsson, An improved method for determination of thiocyanate in plasma and urine. *European Journal of Clinical Chemistry and Clinical Biochemistry* 33 (1995):343–349.

154. H. Z. Li, G. Bai, R. M. Sun, and L. K. Du, Determination of thiocyanate metabolite of sodium nitroprusside in serum by spectrophotometry. *Yao Xue Xue Bao* 28 (1993):854–858.
155. F. Olea and P. Parras, Determination of serum levels of dietary thiocyanate. *Journal of Analytical Toxicology* 16 (1992):258–260.
156. W. A. Dunn and T. J. Siek, A rapid, sensitive, and specific screening technique for the determination of cyanide. *Journal of Analytical Toxicology* 14 (1990):256.
157. M. Falkensson, P. Lundquist, H. Rosling, and B. Sorbo, A simple method for determination of plasma thiocyanate. *Annals of Clinical Biochemistry* 25 (1988):422–423.
158. M. Holzbecher and H. A. Ellenberger, An evaluation and modification of a microdiffusion method for the emergency determination of blood cyanide. *Journal of Analytical Toxicology* 9 (1985):251–253.
159. C. J. Vesey and P. V. Cole, Blood cyanide and thiocyanate concentrations produced by long-term therapy with sodium nitroprusside. *British Journal of Anaesthesia* 57 (1985):148–155.
160. S. Nagashima, Simultaneous reaction rate spectrophotometric determination of cyanide and thiocyanate by use of the pyridine-barbituric acid method. *Analytical Chemistry* 56 (1984):1944–1947.
161. N. Grgurinovich, A colourimetric procedure for the determination of thiocyanate in plasma. *Journal of Analytical Toxicology* 6 (1982):53–55.
162. P. Lundquist, J. Maartensson, B. Sörbo, and S. Öhman, Method for determining thiocyanate in serum and urine. *Clinical Chemistry* 25 (1979):678–681.
163. J. L. Lambert, J. Ramasamy, and J. V. Paukstelis, Stable reagents for the colorimetric determination of cyanide by modified Koenig reactions. *Analytical Chemistry* 47 (1975):916–918.
164. L. S. Bark and H. G. Higson, Investigation of reagents for the colorimetric determination of small amounts of cyanide-I. *Talanta* 11 (1964):471–479.
165. L. S. Bark and H. G. Higson, Investigation of reagents for the colorimetric determination of small amounts of cyanide-II. A proposed method for trace cyanide in waters. *Talanta* 11 (1964):621–631.
166. G. V. L. N. Murty and T. S. Viswanathan, Determination of traces of cyanide. *Analytica Chimica Acta* 25 (1961):293–295.
167. M. Feldstein and N. C. Klendshoj, The determination of cyanide in biological fluids by microdiffusion analysis. *Canadian Journal of Medical Technology* 17 (1955):29–32.
168. E. J. Conway, *Microdiffusion Analysis and Volumetric Error*, 3rd ed. (Crosby Lockwood and Son, Ltd., London, 1950).
169. E. Asmus and H. Garschagen, Use of barbituric acid for the photometric determination of cyanide and thiocyanate. *Fresenius' Zeitschrift fuer Analytische Chemie* 138 (1953):414–422.
170. G. E. Boxer and J. C. Rickards, Determination of thiocyanate in body fluids. *Archives of Biochemistry and Biophysics* 39 (1952):292–300.
171. J. Epstein, Estimation of microquantities of cyanide. *Analytical Chemistry* 19 (1947):272–274.
172. W. N. Aldridge, Estimation of micro quantities of cyanide and thiocyanate. *Analyst* 70 (1945):474–475.
173. W. N. Aldridge, New method for the estimation of microquantities of cyanide and thiocyanate. *Analyst* 69 (1944):262–265.
174. W. König, Verfahren zur darstellung neuer stickstoffhaltiger farbstoffe. *Angewandte Chemie* 69 (1905):115–118.
175. W. A. Groff, F. W. Stemler, A. Kaminskis, H. L. Froehlich, and R. P. Johnson, Plasma free cyanide and blood total cyanide: A rapid completely automated microdistillation assay. *Journal of Toxicology, Clinical Toxicology* 23 (1985):133–163.
176. A. Ganjeloo, G. E. Isom, R. L. Morgan, and J. L. Way, Fluorometric determination of cyanide in biological fluids with p-benzoquinone. *Toxicology and Applied Pharmacology* 55 (1980):103–107.
177. R. L. Morgan and J. L. Way, Fluorometric determination of cyanide in biological fluids with pyridoxal. *Journal of Analytical Toxicology* 4 (1980):78–81.
178. S. Takanashi and Z. Tamura, Fluorometric determination of cyanide by the reaction with pyridoxal. *Chemical & Pharmaceutical Bulletin* 18 (1970):1633–1635.
179. S. L. Tompsett, A note on the determination and identification of cyanide in biologic materials. *Clinical Chemistry* 5 (1959):587–591.
180. T. Toida, S. Tanabe, and T. Imanari, Fluorometric determination of cyanide and thiocyanate by Koenig reaction. *Chemical & Pharmaceutical Bulletin* 29 (1981):3763–3764.
181. G. G. Guilbault and D. N. Kramer, Specific detection and determination of cyanide using various quinone derivatives. *Analytical Chemistry* 37 (1965):1395–1399.
182. J. Y. Dai, R. Yuan, Y. Q. Chai, L. X. An, X. Zhong, Y. Liu, and D. P. Tang, Highly thiocyanate-selective PVC membrane electrode based on lipophilic ferrocene derivative. *Electroanalysis* 17 (2005):1865–1869.
183. J. Y. Dai, Y. Q. Chai, R. Yuan, L. X. An, Y. Liu, X. Zhong, and D. P. Tang, Tricoordinate Schiff base copper(II) complex as neutral carrier for highly selective thiocyanate electrode. *Analytical Letters* 38 (2005):389–400.
184. F. C. Wang, Y. Q. Chai, R. Yuan, C. H. Chen, J. Y. Dai, and L. Xu, A selective membrane electrode for thiocyanate ion based on a bis-taurine-salicylic binuclear copper(II) complex as ionophore. *Chinese Journal of Chemistry* 23 (2005):865–869.
185. D. Shan, C. Mousty, and S. Cosnier, Subnanomolar cyanide detection at polyphenol oxidase/clay biosensors. *Analytical Chemistry* 76 (2004):178–183.
186. M. M. Ardakani, A. A. Ensafi, M. S. Niasari, and S. M. Chahooki, Selective thiocyanate poly(vinyl chloride) membrane based on a 1,8-dibenzyl-1,2,6,8,10,13-hexaazacyclotetradecane-Ni(II) perchlorate. *Analytica Chimica Acta* 462 (2002):25–30.
187. M. R. Ganjali, M. Yousefi, M. Javanbakht, T. Poursaberi, M. Salavati-Niasari, L. Hajiagha-Babaei, E. Latifi, and M. Shamsipur, Determination of SCN^- in urine and saliva of smokers and non-smokers by SCN^- -selective polymeric membrane containing a nickel(II)-azamacrocyclic complex coated on a graphite electrode. *Analytical Sciences* 18 (2002):887–892.
188. A. Abbaspour, M. A. Kamyabi, A. R. Esmailbeig, and R. Kia, Thiocyanate-selective electrode based on unsymmetrical benzoN4 nickel(II) macrocyclic complexes. *Talanta* 57 (2002):859–867.
189. M. K. Amini, S. Shahrokhian, and S. Tangestaninejad, Thiocyanate-selective electrodes based on nickel and iron phthalocyanines. *Analytica Chimica Acta* 402 (1999):137–143.

190. I. G. Casella, Electrooxidation of thiocyanate on copper-modified gold electrode and its amperometric determination by ion chromatography. *Analyst* 123 (1998):1359–1363.
191. Z. Chen and D. B. Hibbert, Simultaneous amperometric and potentiometric detection of inorganic anions in flow systems using platinum and silver/silver chloride electrodes. *Analytica Chimica Acta* 350 (1997):1–6.
192. W. Hu, H. Tao, M. Tominaga, A. Miyazaki, and H. Haraguchi, A new approach for the simultaneous determination of inorganic cations and anions using ion chromatography. *Analytica Chimica Acta* 299 (1994):249–256.
193. E. G. Cookeas and C. E. Efstathiou, Flow injection amperometric determination of thiocyanate and selenocyanate at a cobalt phthalocyanine modified carbon paste electrode. *Analyst* 119 (1994):1607–1612.
194. H. Below, H. Muller, B. Hundhammer, and W. Weuffen, Determination of thiocyanate in serum with ion-selective liquid membrane electrodes. *Pharmazie* 45 (1990):416–419.
195. X. Cai and Z. Zhao, Determination of trace thiocyanate by linear sweep polarography. *Analytica Chimica Acta* 212 (1988):43–48.
196. J. Pasciak and J. Hurek, Catalytic effect of thiocyanate ions and possibility of their determination by pulse polarography with adsorptive concentration on the hanging mercury drop electrode. *Chemia Analityczna* 29 (1984):571–580.
197. J. Du Cailar, J. C. Mathieu-Daude, J. Deschodt, and O. Griffe, Thiocyanate ion plasma levels during perfusion with sodium nitroprusside. *Annales de l'Anesthesiologie Francaise* 17 (1976):519–528.
198. P. Boadas-Vaello, E. Jover, J. Llorens, and J. M. Bayona, Determination of cyanide and volatile alkyl nitriles in whole blood by headspace solid-phase microextraction and gas chromatography with nitrogen phosphorus detection. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* 870 (2008):17–21.
199. K. E. Murphy, M. M. Schantz, T. A. Butler, B. A. Benner, L. J. Wood, and G. C. Turk, Determination of cyanide in blood by isotope-dilution gas chromatography-mass spectrometry. *Clinical Chemistry* 52 (2006):458–467.
200. C. S. Zhang, H. Zheng, O. Y. Jin, S. Z. Feng, and Y. E. C. Taes, Cyanide distribution in human tissue, determined by GC/ECD/HS. *Analytical Letters* 38 (2005):247–256.
201. J. S. Torano and H. J. van Kan, Simultaneous determination of the tobacco smoke uptake parameters nicotine, cotinine and thiocyanate in urine, saliva and hair, using gas chromatography-mass spectrometry for characterisation of smoking status of recently exposed subjects. *Analyst* 128 (2003):838–843.
202. K. Watanabe-Suzuki, A. Ishii, and O. Suzuki, Cryogenic oven-trapping gas chromatography for analysis of volatile organic compounds in body fluids. *Analytical Bioanalytical Chemistry* 373 (2002):75–80.
203. G. Asselborn and R. Wennig, Rapid determination of cyanides in biological material by HS-GC/MS. *Analysemethoden aus dem Arbeitskreis Klinische Toxikologie* 67 (2000):105.
204. S. Tsukamoto, T. Kanegae, Y. Matsumura, S. Uchigasaki, M. Kitazawa, T. Muto, S. Chiba, T. Nagoya, and M. Shimamura, Simultaneous measurement of alcohols and hydrogen cyanide in biological specimens using headspace gas chromatography. *Nippon Hoigaku Zasshi* 48 (1994):336–342.
205. S. H. Chen and S. M. Wu, Electron-capture gas chromatographic determination of cyanide, iodide, nitrite, sulfide, and thiocyanate anions by phase-transfer-catalyzed derivatization with pentafluorobenzyl bromide. *Journal of Analytical Toxicology* 18 (1994):81–85.
206. Z. L. Cardeal, D. Pradeau, and M. Hamon, Determination of HCN by headspace gas chromatography using an improved method of standardization. *Chromatographia* 37 (1993):613–617.
207. M. Yoshida, T. Watabiki, and N. Ishida, The quantitative determination of cyanide by FTD-GC. *Nippon Hoigaku Zasshi* 43 (1989):179–185.
208. P. Jacob, III, C. Savanapridi, L. Yu, M. Wilson, A. T. Shulgin, N. L. Benowitz, B. A. Elias-Baker, S. M. Hall, R. I. Herning, R. T. Jones, and D. P. L. Sachs, Ion-pair extraction of thiocyanate from plasma and its gas chromatographic determination using on-column alkylation. *Analytical Chemistry* 56 (1984):1692–1695.
209. K. Funazo, K. Kusano, H. L. Wu, M. Tanaka, and T. Shono, Trace determination of cyanide by derivatization and flame thermionic gas chromatography. *Journal of Chromatography* 245 (1982):93–100.
210. I. Thomson and R. A. Anderson, Determination of cyanide and thiocyanate in biological fluids by gas chromatography-mass spectrometry. *Journal of Chromatography* 188 (1980):357–362.
211. H. F. de Branander and R. Verbeke, Determination of thiocyanate in tissues and body fluids of animals by gas chromatography with electron-capture detection. *Journal of Chromatography* 138 (1977):131–142.
212. J. C. Valentour, V. Aggarwal, and I. Sunshine, Sensitive gas chromatographic determination of cyanide. *Analytical Chemistry* 46 (1974):924–925.
213. D. Connolly, L. Barron, and B. Paull, Determination of urinary thiocyanate and nitrate using fast ion-interaction chromatography. *Journal of Chromatography B* 767 (2002):175–180.
214. Y. Miura and A. Kawaoi, Determination of thiosulfate, thiocyanate and polythionates in a mixture by ion-pair chromatography with ultraviolet absorbance detection. *Journal of Chromatography A* 884 (2000):81–87.
215. U. Backofen, F. M. Matysik, and G. Werner, Determination of cyanide in microsamples by means of capillary flow injection analysis with amperometric detection. *Analytical and Bioanalytical Chemistry* 356 (1996):271–273.
216. R. Zein, E. Munaf, T. Takeuchi, and T. Miwa, Microcolumn ion chromatography of inorganic UV-absorbing anions using bovine serum albumin as stationary phases. *Analytica Chimica Acta* 335 (1996):261–266.
217. S.-H. Chen, Z.-Y. Yang, H.-L. Wu, H.-S. Kou, and S.-J. Lin, Determination of thiocyanate anion by high-performance liquid chromatography with fluorometric detection. *Journal of Analytical Toxicology* 20 (1996):38–42.
218. C. Bjerregaard, P. Moller, and H. Sorensen, Determination of thiocyanate, iodide, nitrate and nitrite in biological samples by micellar electrokinetic capillary chromatography. *Journal of Chromatography A* 717 (1995):409–414.
219. D. E. C. Cole, J. Evrovski, and R. Pirone, Urinary thiosulfate determined by suppressed ion chromatography with conductimetric detection. *Journal of Chromatography B* 672 (1995):149–154.
220. W. Hu, T. Takeuchi, and H. Haraguchi, Electrostatic ion chromatography. *Analytical Chemistry* 65 (1993):2204–2208.

221. P. Lundquist, B. Backman-Gullers, B. Kågedal, L. Nilsson, and H. Rosling, Fluorometric determination of cyanate in plasma by conversion to 2,4(1H,3H)-quinazolinedione and separation by high-performance liquid chromatography. *Analytical Biochemistry* 211 (1993):23–27.
222. Y. Michigami, K. Fujii, K. Ueda, and Y. Yamamoto, Determination of thiocyanate in human saliva and urine by ion chromatography. *Analyst* 117 (1992):1855–1858.
223. M. Y. Tominaga and A. F. Midio, Modified method for the determination of thiocyanate in urine by ion-exchange chromatography and spectrophotometry. *Revista de Farmacia e Bioquímica da Universidade de São Paulo* 27 (1991):100–105.
224. Y. Michigami, T. Takahashi, F. He, Y. Yamamoto, and K. Ueda, Determination of thiocyanate in human serum by ion chromatography. *Analyst* 113 (1988):389–392.
225. S. Tanabe, M. Kitahara, M. Nawata, and K. Kawanabe, Determination of oxidizable inorganic anions by high-performance liquid chromatography with fluorescence detection and application to the determination of salivary nitrite and thiocyanate and serum thiocyanate. *Journal of Chromatography* 424 (1988):29–37.
226. A. M. Bond, I. D. Heritage, G. G. Wallace, and M. J. McCormick, Simultaneous determination of free sulfide and cyanide by ion chromatography with electrochemical detection. *Analytical Chemistry* 54 (1982):582–585.
227. T. Imanari, S. Tanabe, T. Toida, and T. Kawanishi, High-performance liquid chromatography of inorganic anions using iron(3+) as a detection reagent. *Journal of Chromatography* 250 (1982):55–61.
228. T. Imanari, K. Ogata, S. Tanabe, T. Toida, T. Kawanishi, and M. Ichikawa, Determination of thiocyanate, thiosulfate, sulfite and nitrite by high-performance liquid chromatography coupled with electrochemical detection. *Chemical & Pharmaceutical Bulletin* 30 (1982):374–375.
229. S. Jermak, B. Pranaityte, and A. Padarauskas, Headspace single-drop microextraction with in-drop derivatization and capillary electrophoretic determination for free cyanide analysis. *Electrophoresis* 27 (2006):4538–4544.
230. Z. Glatz, S. Novakova, and H. Sterbova, Analysis of thiocyanate in biological fluids by capillary zone electrophoresis. *Journal of Chromatography A* 916 (2001):273–277.
231. S. E. Manahan and R. Kunkel, Atomic absorption analysis method for cyanide. *Analytical Letters* 6 (1973):547–553.
232. S. Chattaraj and A. K. Das, Indirect determination of thiocyanate in biological fluids using atomic absorption spectrometry. *Spectrochimica Acta, Part B* 47B (1992):675–680.
233. G. E. Boxer and J. C. Rickards, The metabolism of the carbon of cyanide and thiocyanate. *Archives of Biochemistry and Biophysics* 39 (1952):7–26.
234. Y. Seto, Oxidative conversion of thiocyanate to cyanide by oxy-hemoglobin during acid denaturation. *Archives of Biochemistry and Biophysics* 321 (1995):245–254.
235. Y. Seto, False cyanide detection. *Analytical Chemistry* 74 (2002):134A–141A.
236. C. J. Vesey and J. Wilson, Red cell cyanide. *Journal of Pharmacy and Pharmacology* 30 (1978):20–26.
237. B. Ballantyne, In vitro production of cyanide in normal human blood and the influence of thiocyanate and storage temperature. *Clinical Toxicology* 11 (1977):173–193.
238. Y. Seto, Stability and spontaneous production of blood cyanide during heating. *Journal of Forensic Sciences* 41 (1996):465–468.
239. M. Feldstein and N. C. Klendshoj, The determination of volatile substances by microdiffusion analysis. *Journal of Forensic Sciences* 2 (1957):39–58.
240. A. Cruz-Landeira, M. Lopez-Rivadulla, L. Concheiro-Carro, P. Fernandez-Gomez, and M. J. Tabernero-Duque, A new spectrophotometric method for the toxicological diagnosis of cyanide poisoning. *Journal of Analytical Toxicology*, 24 (2000):266–270.
241. G. Nota, C. Improta, and V. R. Miraglia, Determination of cyanides and thiocyanates in water as cyanogen bromide by headspace gas chromatography. *Journal of Chromatography* 242 (1982):359–363.
242. R. S. Danchik and R. F. Boltz, Indirect atomic absorption photometric methods for the determination of cyanide. *Analytica Chimica Acta* 49 (1970):567–569.
243. D. Gao, J.-Z. Li, Y. Ru-Qin, and G.-D. Zheng, Metalloporphyrin derivatives as neutral carriers for PVC membrane electrodes. *Analytical Chemistry* 66 (1994):2245–2249.
244. M. J. Segui, J. Lizondo-Sabarter, R. Martinez-Manez, F. San-cenon, and J. Soto, Linear polyamines as carriers in thiocyanate-selective membrane electrodes. *Talanta* 68 (2006):1182–1189.
245. C. V. Barber and V. R. Pratt, *Sullied Seas. Strategies for Combating Cyanide Fishing in Southeast Asia and Beyond* (World Resources Institute, International Marinelife Alliance, Washington, DC, 1997).
246. B. Fouillet, M. Odoul, R. Chambon, and P. Chambon, Specific determination of cyanide in water by GC/ECD as cyanogen chloride. *Journal Francais d'Hydrologie* 23 (1992):161–169.
247. S. H. Chen, H. L. Wu, M. Tanaka, T. Shono, and K. Funazo, Simultaneous gas chromatographic determination of iodide, nitrite, sulfide, and thiocyanate anions by derivatization with pentafluorobenzyl bromide. *Journal of Chromatography* 396 (1987):129–137.
248. S. Kage, T. Nagata, and K. Kudo, Determination of thiosulfate in body fluids by GC and GC/MS. *Journal of Analytical Toxicology* 15 (1991):148–150.
249. J. O. Egekeze and F. W. Oehme, Direct potentiometric method for the determination of cyanide in biological materials. *Journal of Analytical Toxicology* 3 (1979):119–124.
250. P. Houeto, J. R. Hoffman, M. Imbert, P. Levillain, and F. J. Baud, Relation of blood cyanide to plasma cyanocobalamin concentration after a fixed dose of hydroxocobalamin in cyanide poisoning. *Lancet* 346 (1995):605–608.
251. C. J. Vesey, P. V. Cole, and P. J. Simpson, Cyanide and thiocyanate concentrations following sodium nitroprusside infusion in man. *British Journal of Anaesthesia* 48 (1976):651–660.
252. M. Ansell and F. A. S. Lewis, Review of cyanide concentrations found in human organs. Survey of literature concerning cyanide metabolism, "normal," nonfatal, and fatal body cyanide levels. *Journal of Forensic Medicine* 17 (1970):148–155.
253. A. S. Curry, *Poison Detection in Human Organs*, 3rd ed. (Charles C. Thomas Publishers, Springfield, IL, 1976).
254. J. E. Bright, R. H. Inns, N. J. Tuckwell, and T. C. Marrs, The effect of storage upon cyanide in blood samples. *Human & Experimental Toxicology* 9 (1990):125–129.

255. F. Chikasue, M. Yashiki, T. Kojima, T. Miyazaki, I. Okamoto, M. Ohtani, and K. Kodama, Cyanide distribution in five fatal cyanide poisonings and the effect of storage conditions on cyanide concentration in tissue. *Forensic Science International* 38 (1988):173–183.
256. C. J. Vesey and R. M. Langford, Stabilization of blood cyanide. *Journal of Analytical Toxicology* 22 (1998):176–178.
257. S. Goenechea, Hydrogen cyanide: Losses in samples of stored blood. *Zeitschrift fuer Rechtsmedizin* 88 (1982):97–101.
258. B. Ballantyne, Changes in blood cyanide as a function of storage time and temperature. *Journal of the Forensic Science Society* 16 (1976):305–310.
259. H. Chandra, B. N. Gupta, S. K. Bhargava, C. S. H., and M. P. N., Chronic cyanide exposure—A biochemical and industrial hygiene study. *Journal of Analytical Toxicology* 4 (1980):161–165.
260. I. S. Symington, R. A. Anderson, I. Thomson, J. S. Oliver, W. A. Harland, and J. W. Kerr, Cyanide exposure in fires. *Lancet* 2 (1978):91–92.
261. M. S. Levine and E. P. Radford, Occupational exposures to cyanide in Baltimore fire fighters. *Journal of Occupational Medicine* 20 (1978):53–56.
262. R. Pojer, J. B. Whitfield, V. Poulos, I. F. Eckhard, R. Richmond, and W. J. Hensley, Carboxyhemoglobin, cotinine, and thiocyanate assay compared for distinguishing smokers from non-smokers. *Clinical Chemistry* 30 (1984):1377–1380.
263. E. R. Dalferes, Jr., L. S. Webber, B. Radhakrishnamurthy, and G. S. Berenson, Continuous-flow (AutoAnalyzer I) analysis for plasma thiocyanate as an index to tobacco smoking. *Clinical Chemistry* 26 (1980):493–495.
264. S. S. M. Hassan, W. H. Mohmoud, A. Elgazwy, and N. M. Badawy, A novel potentiometric membrane sensor based on aryl palladium complex for selective determination of thiocyanate in the saliva and urine of cigarette smokers. *Electroanalysis* 18 (2006):2070–2078.
265. A. B. Bendtsen and E. H. Hansen, Spectrophotometric flow injection determination of trace amounts of thiocyanate based on its reaction with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol and dichromate: assay of the thiocyanate level in saliva from smokers and non-smokers. *Analyst* 116 (1991):647–651.